CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER: 20-837

PHARMACOLOGY REVIEW(S)

Division of Pulmonary Drug Products

Review of Pharmacology/Toxicology Data MAR - 8 1999

Review: Chemistry Consult

Reviewer: VEWhitehurst

Review Completion Date: March 4, 1999

Information to be Conveyed to the Sponsor: Yes, via chemist

HFD: HFD 570

NDA: NDA 20-837

Sponsor: Sepracor
111 Locke Drive
Marlborough, MA 01752

Drug: Xopenex (Levalbuterol HCl) Inhalation Solution

Category: Beta agonist

Indication: Treatment or prevention of [redacted] bronchospasm in patients with reversible obstructive airway disease and attacks of bronchospasm.

Administration: Inhalation

Composition and Dosage Forms:

Components	**1.25 mg (mg/3ml)**	**0.63 mg (mg/3ml)**	
Levalbuterol HCl			
Sodium chloride			
Sulfuric acid,			

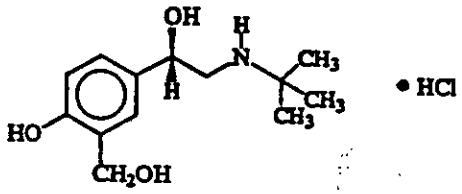
USAN: Levalbuterol Hydrochloride

Chemical name: (R)-α1-[[(1,1-dimethylethyl)amino]methyl]-4-hydroxy-1,3-benezenedimethanol hydrochloride

Molecular Weight: 275.8

Cas Registry #: 50293-90-8

Chemical Structure:



Related IND/NDA: IND [redacted] and IND [redacted]

Introduction and History:
The chemistry consult concerns the maximum allowable levels of the degradants / impurities in the drug products as follows:

Impurities/Degradation Products	**Proposed Specifications**
Albuterol aldehyde	[redacted]

Recommendation:
We concur with the proposed specifications for albuterol aldehyde in the drug product. The specifications proposed by the sponsor are consistent with the levels of albuterol aldehyde proposed for Combivent (Ipratropium bromide and albuterol sulfate), IND [redacted] (See IND [redacted] pharmacology review dated Sept 9, 1996 which recommended that the levels of albuterol aldehyde be as low as possible because of its mutagenic potential, preferably [redacted] however, if [redacted] was not

practicable, then a limit of [redacted] was deemed acceptable.) Refrigeration, nitrogen and overwrapping may be recommended by the chemist to inhibit degradation and keep the impurity as low as possible.

Conclusion:
Our recommendation should be conveyed to the sponsor via the chemist.

/S/

Virgil Whitehurst 3-8-99
Pharmacologist

CC:NDA 20-837
HFD 570/div file
HFD 570/Shah
HFD 570/Whitehurst
HFD 570/Huff /S/ 3-8-99
HFD 570/Jani

APPEARS THIS WAY
ON ORIGINAL

/S/

Division of Pulmonary Drug Products

Review of Pharmacology/Toxicology Data

FEB 11 1999

Review : Revised labelling

Review Completion Date: January 21, 1999

Information to be conveyed to the Sponsor: Yes

HFD: HFD 570

NDA: NDA 20-837

Sponsor: Sepracor

Drug: Xopenex (Levalbuterol HCl) Inhalation Solution

Category: Beta Adrenergic Agonist

Indication: Treatment or prevention of [redacted] bronchospasm in patients with reversible obstructive airway disease and attacks of bronchospasms.

Administration: Inhalation

Review of Revised labelling:
The preclinical labelling for Xopenex should be revised as follows:

Clinical Pharmacology Section:
Lines 35-40 should be removed from labelling. The in vitro data is not clinically relevant.

Preclinical Section:
Lines 54-62 should be removed. The data from the pharmacology and in vitro studies are not clinically relevant. The data from these studies may be reconsidered for inclusion in the labelling provided clinical relevance is shown.

Lines 64-69 should be revised as follows:
Intravenous studies in rats with racemic albuterol sulfate have demonstrated that albuterol crosses the blood-brain barrier and reaches brain concentrations amounting to approximately 5.0 % of the plasma concentrations. In structures outside the the blood–brain barrier (pineal and pituitary glands), albuterol concentrations were found to be 100 times those in whole brain.

Lines 71-75 should be revised as follows:
Studies in laboratory animals (minipigs, rodents and dogs) have demonstrated the occurrence of cardiac arrhythmias and sudden death (with histologic evidence of myocardial necrosis) when beta agonists and methylxanthines are administered concurrently. The clinical significance of these findings is unknown.

Carcinogenesis, Mutagenesis andImpairment of Fertility:
No carcinogenesis or impairment of fertility studies have been carried out with levalbuterol HCl. However, racemic albuterol sulfate has been evaluated for its carcinogenic potential and its ability to impair fertility.

In a 2-year study in Sprague-Dawley rats, racemic albuterol sulfate caused a significant dose-related increase in the incidence of benign leiomyomas of the mesovarium at and above dietary doses of 2 mg/kg (approximately 2 times the maximum recommended daily inhalation dose of levalbuterol HCl for adults on a mg/m^2 basis). In another study this effect was blocked by the coadministration of propranolol, a non-selective beta-adrenergic antagonist. In an 18-month study in CD-1 mice, racemic albuterol sulfate showed no evidence of tumorigenicity at dietary doses up to 500 mg/kg (approximately 270 times the maximum recommended daily inhalation dose of levalbuterol HCl for adults on a mg/m^2 basis). In a 22-month study in the Golden hamster, racemic albuterol sulfate showed no evidence of tumorigenicity at dietary doses up to 50 mg/kg (approximately 35 times the maximum recommended daily inhalation dose of levalbuterol HCl for adults on a mg/m^2 basis).

Levalbuterol HCl was not mutagenic in either the Ames test or the CHO/HPRT mammalian gene mutation assay. Although levalbuterol HCl has not been tested for clastogenicity, racemic albuterol sulfate was not clastogenic in a human peripheral lymphocyte assay or in an AH1 strain mouse micronucleus assay.

Reproduction studies in rats using racemic albuterol sulfate demonstrated no evidence of impaired fertility at oral doses up to 50 mg/kg (approximately 55 times the maximum recommended daily inhalation dose of levalbuterol HCl for adults on a mg/m^2 basis).

Teratogenic Effects–Pregnancy Category C: A reproduction study in New Zealand white rabbits demonstrated that levalbuterol HCl was not teratogenic when administered orally at doses up to 25 mg/kg (approximately 110 times the maximum recommended daily inhalation dose of levalbuterol HCl for adults on a mg/m^2 basis). However, racemic albuterol sulfate has been shown to be teratogenic in mice and rabbits. A study in CD-1 mice given racemic albuterol sulfate subcutaneously showed cleft palate formation in 5 of 111 (4.5%) fetuses at 0.25 mg/kg (less than the maximum recommended daily inhalation dose of levalbuterol HCl for adults on a mg/m^2 basis) and in 10 of 108 (9.3%) fetuses at 2.5 mg/kg (approximately equal to the maximum recommended daily inhalation dose of levalbuterol HCl for adults on a mg/m^2 basis). The drug did not induce cleft palate formation when administered at a subcutaneous dose of 0.025 mg/kg (less than the maximum recommended daily inhalation dose of levalbuterol HCl for adults on a mg/m^2 basis). Cleft palate also occurred in 22 of 72 (30.5%) fetuses from females treated subcutaneously with 2.5 mg/kg isoproterenol (positive control). A reproduction study in Stride Dutch rabbits revealed cranioschisis in 7 of 19 (37%) fetuses when racemic abuterol sulfate was administered orally at 50 mg/kg (approximately 110 times the maximum recommended daily inhalation dose of levalbuterol HCl for adults on a mg/m^2 basis).

A study in which pregnant rats were dosed with radiolabeled racemic albuterol sulfate demonstrated that drug-related material is transferred from the maternal circulation to the fetus.

There are no adequate and well-controlled studies in pregnant women. Because animal studies are not always predictive of human response, levalbuterol HCl should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus.

During marketing of racemic albuterol sulfate various congenital anomalies, including cleft palate and limb defects, have been reported in the offspring of patients being treated with albuterol. Some of the mothers were taking

multiple medications during their pregnancies. Because no consistent pattern of defects can be discerned, a relationship between albuterol use and congenital anomalies has not been established.

Overdosage :
The intravenous median lethal dose of levalbuterol HCl in mice is approximately 66 mg/kg (approximately 70 times the maximum recommended daily inhalation dose of levalbuterol HCl for adults on a mg/m^2 basis). The inhalation median lethal dose has not been determined.

Calculations for levalbuterol HCl are listed below:

Drug:
Xopenex

	Age	mg/dose	# daily Doses	mg/day	kg	mg/kg	Factor	mg/m^2
Adult	>12	1.25	3	3.75	50	0.08	37	2.78

	Route	Mg/kg/d	Conv. Factor	mg/m^2	Dose Ratio Adults	Rounded Dose Ratio Adults
Carcinogenicity:						
mouse			3	0	—	—
mouse			3	0	—	—
mouse	Dietary	500	3	1500	540.541	540*
rat	Dietary	2	6	12	4.31	4.0*
hamster	Dietary	50	4	200	71.9	70
Reproduction and Fertility:						
rat	Oral	50	6	300	108.108	110*
rat			6	0	—	—
rat			6	0	—	—
extra			—	—	—	—
Teratogenicity:						
mouse	sc	0.25	3	0.75	0.27027	¼*
mouse	sc	0.025	3	0.075	0.0270	¼*
rabbit	Oral	25	12	300	108.108	110
rabbit		50	12	0	215.8—	220*

mouse	sc	2.5	3	7.5	2.7027		3*
Overdosage:							
mouse	iv	66	3	198	71.3514		70
mouse			3	0	—		—
rat			6	0	—		—
rat			6	0	—		—
Other: (Describe studies here)							
rat			6	0	—		—
rat			6	0	—		—
mouse			3	0	—		—
mouse			3	0	—		—
extra			—	—	—		—

* Calculations in labeling are ½ of the dose ratios based on the fact that R-albuterol is 50% of the racemic mixture of albuterol sulfate.

Recommendation : Please send labeling revisions to the sponsor .

/S/

Virgil Whitehurst
Pharmacologist 2-11-99

CC : Div File
HFD-570/Huff /S/ 2-11-99
HFD-570/Whitehurst
HFD-570/Jani
HFD-570/Shah
HFD-570/Nicklas

APPEARS THIS WAY
ON ORIGINAL

JUN 1 0 1998

Division of Pulmonary Drug Products

Review of Pharmacology/Toxicology Data

Chemistry Consult

Reviewer : VEWhitehurst

Review Completion Date : June 10, 1998

Information to be Conveyed to the Sponsor : Yes

HFD : HFD 570

NDA : NDA 20-837

Sponsor : Sepracor
111 Locke Drive
Marlborough, MA 01752

Drug : [redacted](Levalbuterol HCL) Inhalation Solution

Category : Beta agonist

Indication : Treatment or prevention of [redacted] bronchospasm in patients [redacted] years of age and older with reversible obstructive airway disease [redacted]

Administration : Inhalation

Composition and Dosage Forms :

Components	**1.25 mg (mg/ 3ml)**	**0.63 mg (mg/3ml)**	[redacted]

Levalbuterol HCL (mg)
Sodium chloride
Sulfuric acid,

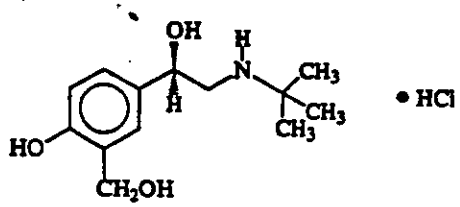
USAN : Levalbuterol Hydrochloride

Chemical name : (R)-α1-[[(1,1-dimethylethyl(amino]methyl]-4-hydroxy-1,3benezenedimethaniol hydrochloride

Molecular Weight : 275.8

Cas Registry # : 50293-90-8

Chemical Structure :



Related IND/NDA : IND

Introduction and History :
The chemistry consult concerns the maximum allowable levels of the degradants and impurities as follows:

Drug substance:

Degradants/impurities	Proposed Specifications
Monoethyl ether albuterol	

	Albuterol aldehyde	[redacted]
Drug product	5-Hydro-albuterol	
	Albuterol aldehyde	

*= Not more than

The chemist wanted to know what levels of these degradants/impurities that are allowable in Levalbuterol Inhalation Solution.

Recommendation :

We recommend a limit of [redacted] of albuterol aldehyde for both the drug substance and drug product. This recommendation is consistent with our recommendation for IND [redacted] which also recommended that the levels of albuterol aldehyde be as low as possible. Refrigeration, nitrogen and overwrapping should be recommended by the chemist to keep the impurity as low as possible.

We recommend that the level of monoethyl ether albuterol for the drug substance be limited to [redacted]

The maxim level of 5-hydro albuterol in the drug product proposed by the sponsor is acceptable.

[redacted]
/S/
Virgil Whitehurst 6/10/98
Pharmacologist

[redacted]
/S/
6/10/98

CC :
HFD : 570/div file
HFD: 570/HS/Team leader
HFD:570/VS/ Chemistry reviewer
HFD: 570/ VW/ Pharmacology reviewer
HFD:570/PJ/Project manager

APPEARS THIS WAY
ON ORIGINAL

APR 21 1998

Division of Pulmonary Drug Products

Review of Pharmacology/Toxicology Data

Chemistry Consult

Reviewer : VEWhitehurst

Review Completion Date : April 21, 1998.

Information to be Conveyed to the Sponsor : Yes

HFD : HFD 570

NDA : NDA 20-837

Sponsor : Sepracor
111 Locke Drive
Marlborough, MA 01752

Drug : [redacted] (Levalbuterol HCL) Inhalation Solution

Category : Beta agonist

Indication : Treatment or prevention of [redacted] bronchospasm in patients [redacted] years of age and older with reversible obstructive airway disease and [redacted]

Administration : Inhalation

Composition and Dosage Forms :

Components	**1.25 mg (mg/ 3ml)**	**0.63 mg (mg/3ml)**	[redacted]

Levalbuterol HCL (mg)
Sodium chloride
Sulfuric acid,

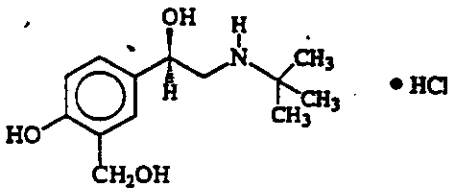
USAN : Levalbuterol Hydrochloride

Chemical name : (R)-α1-[[(1,1-dimethylethyl(amino]methyl]-4-hydroxy-1,3benezenedimethaniol hydrochloride

Molecular Weight : 275.8

Cas Registry # : 50293-90-8

Chemical Structure :



Related IND/NDA : IND

Introduction and History :

The chemistry consult concerns the maximum allowable levels of the degradant, albuterol aldehyde. The sponsor proposed not more than w/w of albuterol aldehyde for the drug substance and not more than w/w for the drug product. The chemist wanted to know what levels of albuterol aldehyde were allowable.

Recommendation :

We recommend a limit of < [redacted] w/w of albuterol aldehyde for both the drug substance and drug product. This recommendation is consistent with our recommendation for IND [redacted] which also recommended that the levels of albuterol aldehyde be as low as possible. Refrigeration, nitrogen and overwrapping should be recommended by the chemist to keep the impurity as low as possible.

/S/

Virgil Whitehurst
Pharmacologist 4-21-98

/S/

CC :
HFD : 570/div file
HFD: 570/HS/Team leader
HFD:570/VS/ Chemistry reviewer
HFD: 570/ VW/ Pharmacology reviewer

APPEARS THIS WAY
ON ORIGINAL

DIVISION OF PULMONARY DRUG PRODUCTS
REVIEW AND EVALUATION OF PHARMACOLOGY AND TOXICOLOGY DATA
Review # 2

IND No. [redacted] **Serial No.** 037 **Submission Date:** 09 OCT 95

Information to be Conveyed to Sponsor: Yes ( ), No (✓)

Reviewer: W. Mark Vogel, Ph.D. **Date Review Completed:** 09 SEP 96

Sponsor: Boehringer Ingelheim, Ridgefield, CN

Drug Name: Combivent® (ipratropium bromide and albuterol sulfate)

Chemical Formulae, Structures, and Molecular Weights: See figure 1, page 3.

Related INDs & NDAs:

IND [redacted] Combivent, albuterol/ipratropium aerosol
NDA 20-291, Boehringer, Combivent, albuterol/ipratropium aerosol
NDA 20-228, Boehringer, Atrovent, ipratropium inhalation solution
NDA 20-393, Boehringer, Atrovent ipratropium 0.03% nasal spray
NDA 20-394, Boehringer, Atrovent ipratropium 0.06% nasal spray
NDA 19-243, Schering, Proventil, 0.083% albuterol inhalation solution
NDA 19-773, Glaxo, Ventolin, 0.083% albuterol inhalation solution

Class: *Ipratropium* - muscarinic cholinergic antagonist, bronchodilator
Albuterol - selective β_2-adrenergic agonist, bronchodilator

Indication: Bronchodilator for COPD (chronic bronchitis or emphysema)

Clinical Formulation: Inhalation solution, single dose vial, [redacted] mg albuterol sulfate, [redacted] mg ipratropium bromide [redacted] mL

Route of Administration: Oral inhalation via nebulizer

Previous Review(s), Date(s) and Reviewer(s):

Original IND [redacted] Pharm/Tox review, 10 MAY 90, Y.S. Choi
Original NDA 20-291 Pharm/Tox review, 09 NOV 93, C.J. Sun

Preclinical Studies Submitted and Reviewed in this IND:

Study	Report #	Vol	Page
Acute toxicity studies with ipratropium and albuterol degradants:			
Ipratropium degradants, acute mouse intravenous toxicity	U95-3051	1	86
Albuterol degradants, acute mouse oral gavage toxicity	U95-3057	1	268
Bacterial reverse mutational (Ames) assays with ipratropium and albuterol degradants:			
Ortho-hydroxy-ipratropium (BIRJ 420 Br), bacterial mutagenicity	U95-3052	1	104
Meta-hydroxy-ipratropium (BIRJ 416 Br), bacterial mutagenicity	U95-3053	1	139
Para-hydroxy-ipratropium (BIRT 109 Br), bacterial mutagenicity	U95-3054	1	172
Hydroxy-albuterol (BIRG 695 BS), bacterial mutagenicity	U95-3055	1	206
Albuterol aldehyde (BIRG 597 BS), bacterial mutagenicity	U95-3056	1	239
Additional genetic toxicology studies of albuterol aldehyde (BIRG 597 BS):			
Albuterol aldehyde, mouse lymphoma mutagenicity	U95-3119	1	304
Albuterol aldehyde, human lymphocyte *in vitro* cytogenetics	U95-3120	1	336
Albuterol aldehyde, CHO/HGPRT mammalian *in vitro* mutagenicity	U95-3121	1	379
Albuterol aldehyde, mouse micronucleus assay	U95-3124	1	457
In vitro genetic toxicology assays of Combivent unit dose vial solution:			
Combivent UDV solution, bacterial mutagenicity	U95-3122	1	407
Combivent UDV solution, mouse lymphoma mutagenicity	U95-3123	1	431

Studies Not Reviewed in this IND: None

Studies Previously Reviewed: None

Note: Portions of this review were excerpted directly from the sponsor's submission.

Redacted 1

pages of trade secret and/or confidential commercial information

TOXICOLOGY

ACUTE ORAL TOXICITY:

Acute Intravenous Toxicity of Ipratropium and its Derivatives
Boeheringer Ingelheim report U95-3051, 22 FEB 95, pg 86.

Study Dates: 03 OCT 94 to 17 OCT 94
Testing Lab: Boehringer Ingelheim Pharmaceuticals, Investigative Toxicology Section, Ridgefield, CT.
Test Articles:
Ipratropium bromide, lot 230300, batch RM-1335,
BIRJ 420 Br (ortho-hydroxy ipratropium), lot PDL A/94;2372-115-1,
BIRJ 416 Br (meta-hydroxy ipratropium), lot PDL A/94;2372-89-1,
BIRT 109 Br (para-hydroxy ipratropium), lot PDL A/94;2212-60, [redacted]

GLP: The study was accompanied by a signed GLP statement.

Methods: CD-1 mice [Crl:CD(ICRBR) VAF$^+$] were allotted to four groups (5/sex/group), each treated with a single 11 mg/kg i.v. dose of one of the test articles. The vehicle was 0.9% NaCl adjusted to pH3.5; there was no vehicle control group. The 11 mg/kg dose was considered a minimal lethal dose of ipratropium bromide based on previous i.v. toxicity studies (0177/U, U74-1005, U75-0155). Body weight was measured on days 1, 8, and 15. Clinical observations were recorded pre-dose, and 0.25, 1, 2, and 4 hours post-dose on day-1, and daily thereafter. A gross necropsy was performed on day 15; there was no microscopic examination.

Results: Relevant findings are outlined in the table below. *Deaths* occurred in all treatment groups, with a slightly higher incidence in the meta-hydroxy ipratropium group. All deaths occurred on day-1 during or immediately after dosing. *Clinical Signs* differed somewhat among groups. Decreased motor activity was seen only with ipratropium. Convulsions occurred in all of the hydroxy ipratropium groups. Purple tipped tail, suggesting hypoxia, occurred only with para-hydroxy ipratropium. *Body Weights* in survivors did not differ among groups. *Gross Pathology*: The only treatment-related findings were red foci in lungs, interpreted as agonal hemorrhages.

n=5/group	Ipratropium		o-Hydroxy Ipratropium		m-Hydroxy Ipratropium		p-Hydroxy Ipratropium	
	♂	♀	♂	♀	♂	♀	♂	♀
Mortality	1	0	0	2	3	1	1	0
Decreased motor activity	2	0	0	0	0	0	0	0
Convulsions	0	0	0	3	2	1	2	1
Purple-tipped tail	0	0	0	0	0	0	1	2

of acute toxicity. Thus, albuterol aldehyde (LD_{50} < 2500 mg/kg) appears to have greater acute toxicity than hydroxyalbuterol (LD_{50} ≥ 5000 mg/kg). Neither appears to have greater acute toxicity than albuterol (literature LD_{50} range 1900-4700 mg/kg reported in Registry of Toxic Effects of Chemical Substances).

GENETIC TOXICOLOGY

Bacterial Reverse Mutational Assays with Ipratropium and Albuterol Degradants
Boeheringer Ingelheim reports: U95-3052, pg 104; U95-3053, pg 139; U95-3054, pg 172; U95-3055, pg 206; U95-3056, pg 239.

These reports are reviewed together; the same experimental protocol was used for all.

Study Dates: 14 SEP 94 to 19 JAN 95
Testing Lab: Boehringer Ingelheim Pharmaceuticals, Investigative Toxicology Section, Ridgefield, CT.
Test Articles:
BIRJ 420 Br (ortho-hydroxy ipratropium), lot PDL A/94;2372-115-1,
BIRJ 416 Br (meta-hydroxy ipratropium), lot PDL A/94;2372-89-1,
BIRT 109 Br (para-hydroxy ipratropium), lot PDL A/94;2212-60,
BIRG 695 BS (hydroxy-albuterol), lot 2439-147-1,batch B/94,
BIRG 597 BS (albuterol aldehyde),lot 2439-98-1, batch E/94,
[redacted]

GLP: The studies were accompanied by signed GLP statements. The following deviations from GLP do not invalidate the studies: No analyses of test articles were done for the final vehicle solution. Stability of test article in solution was not determined; all dosing solutions were used within 4 hr of preparation.

Methods: Each compound was tested, with and without metabolic activation with liver S9 fraction from Aroclor-1254-induced rats. The [redacted] method was used with *Salmonella typhimurium* strains TA1535, TA1537, TA98, TA100, and *Escherichia coli* WP2 uvrA (pKM101). The bacterial strains' genetic phenotypes were verified by appropriate tests. Concentrations of test articles (see Results) were based on preliminary cytotoxicity tests in strain TA100 only. Triplicate plates at each concentration were incubated for 48 hours. Colonies were counted manually or with an automated counter. The guidelines for a positive response were a reproducible, dose-related increase in mean revertant numbers. For strain TA100, which has a high baseline revertant mutation rate, an increase in mean revertant numbers to 2 times the solvent control was considered positive; for the other strains the criteria were a 3-fold increase over solvent control or an absolute number ≥ 20, whichever was greater. Test articles were dissolved in DMSO, which was used as the solvent control. Positive controls for the various strains with and without metabolic activation by liver S9 fraction were as follows:

Acute Oral Toxicity of Albuterol Derivatives
Boeheringer Ingelheim report: U95-3057, 17 APR 95, pg 268.

Study Dates: 14 SEP 94 to 19 JAN 95
Testing Lab: Boehringer Ingelheim Pharmaceuticals, Investigative Toxicology Section, Ridgefield, CT.
Test Articles:
BIRG 695 BS (hydroxy-albuterol), lot 2439-147-1,batch B/94 [redacted]
BIRG 597 BS (albuterol aldehyde),lot 2439-98-1, batch E/94,

Methods: CD-1 mice [Crl:CD(ICRBR) VAF$^+$] were allotted to seven groups (5/sex/group): vehicle (1% carboxymethyl cellulose, 0.2% Tween 80), 1000, 2500, or 5000 mg/kg of hydroxy-albuterol, and 1000, 2500, or 5000 mg/kg of albuterol aldehyde. Drug was given as a single dose by oral gavage. After the first 3/sex/group were treated the vehicle was changed due to poor solubility and high viscosity. Dosing volume was increased and 10% ethanol was added; drug was given in two divided doses given ~2 hours apart. An additional 2 animal /sex/group were dosed in any group where mortality or significant morbidity was observed up to 48 hours after dosing on day-1. Clinical observations were made immediately pre-dose, at 0.25 and 1 hour after the first dose, at 1 and 2 hours after the second dose, and once daily thereafter. Body weight was recorded on days 1, 7 or 8 and 13. A gross necropsy was performed on day 15 without microscopic examination.

Results: *Mortality:* Treatment-related mortality is summarized in the table below; animals were found dead on days 1 or 2. *Clinical Signs:* For hydroxy-albuterol ptosis, rough coat, gasping, decreased motor activity and stained urine were observed on day 1 in several animals at 5000 mg/kg. For albuterol aldehyde decreased motor activity, ataxia, rough coat, gasping, ptosis, and coolness to touch were observed at 2500, and 5000 mg/kg. For both agents, most of the survivors appeared normal by day 2. *Body Weight:* There were no drug-related changes in body weights. *Gross Pathology:* For hydroxy-albuterol, mucosal red discoloration of the stomach and red parenchymal discoloration of the lungs were observed in one high-dose male that died on day-1. Pale parenchyma of the kidneys was seen in one high-dose female. For albuterol aldehyde, treatment-related changes were found in all 12 animals that died on day-1 and in 7/9 that died on day-2. The most frequent findings were mucosal red discoloration of the stomach and perioral yellow discoloration of the skin. Findings with lower incidence included distention of the stomach, red discoloration of the colon contents and of the intestinal mucosa. These findings implicate the gastrointestinal tract as the primary target

Acute Mortality of Albuterol Derivatives

mg/kg	Hydroxy-albuterol		Albuterol Aldehyde	
	♂	♀	♂	♀
0	0/5	0/5		
1000	0/5	0/5	0/5	1/5
2500	0/5	0/5	5/5	5/5

Strain	Positive Controls	
	Without S9	With S9
TA1535	sodium azide 10 μg/plate	2-aminoanthracene 1 μg/plate
TA1537	9-aminoacridine 50 μg/plate	2-aminoanthracene 2 μg/plate
TA98	2-nitrofluorene 1 μg/plate	2-aminoanthracene 1 μg/plate
TA100	sodium azide 10 μg/plate	2-aminoanthracene 1 μg/plate
WP2 uvrA pKM101	N-ethyl-N'-nitro-N-nitrosoguanidine 0.5-1.0 μg/plate	2-aminoanthracene 2-4 μg/plate

Results:

Ipratropium Derivatives: The ortho-, para- and meta-hydroxy ipratropium derivatives were each tested at 0.5, 1, 2, 3, 4, and 5 mg/plate, with or without S9. The meta- and para-hydroxy derivatives exhibited no toxicity and had no effect on revertant colony number. Ortho-hydroxy-ipratropium exhibited marginal mutagenic activity in the E. coli WP2 strain, without metabolic activation, as shown in figure 2. The criterion for a positive response in this strain was a 3-fold increase in revertant colony number compared to the concurrent vehicle control. In the first trial, revertant colony number was clearly increased from a control value of 54 to a peak of 276 (5.1x) at 4 mg/plate. In a repeat trial, revertant colonies increased from 62 to 185 (3.0x) at 3 mg/plate. In a third trial, revertant colonies increased from 65 to 161 (2.45x) at 5 mg/plate. All of the peak values were greater than the highest historic control value of 150 revertant colonies. In the first two runs the peak number of mutant colonies in the presence of test article exceeded the number of mutant colonies seen with the positive control (213, 160, and 273; respectively, in runs 1,2, and 3). The effect appears to be dose-related, with a possibility of decreased colony number at higher doses due to toxicity. Mutations in this strain imply a base-pair substitution mechanism The sponsor concluded that ortho-hydroxy- ipratropium is mutagenic in this assay. The sponsor argues that the relatively high doses needed to induce mutations, and the absence of activity under conditions of metabolic activation, suggest that there is no genetic hazard *in vivo*. This argument is tenuous. The test is designed to identify mutagens not for dose-response risk assessment. The compound may be metabolized *in vivo* to a non-muta-

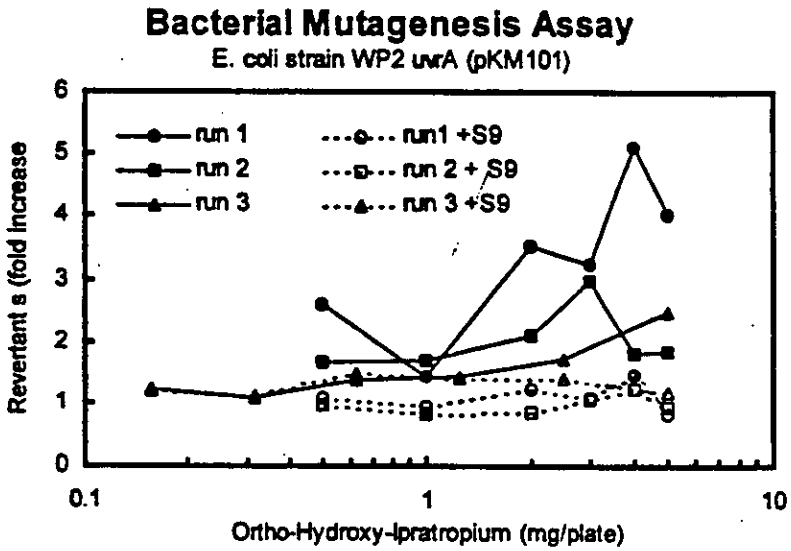


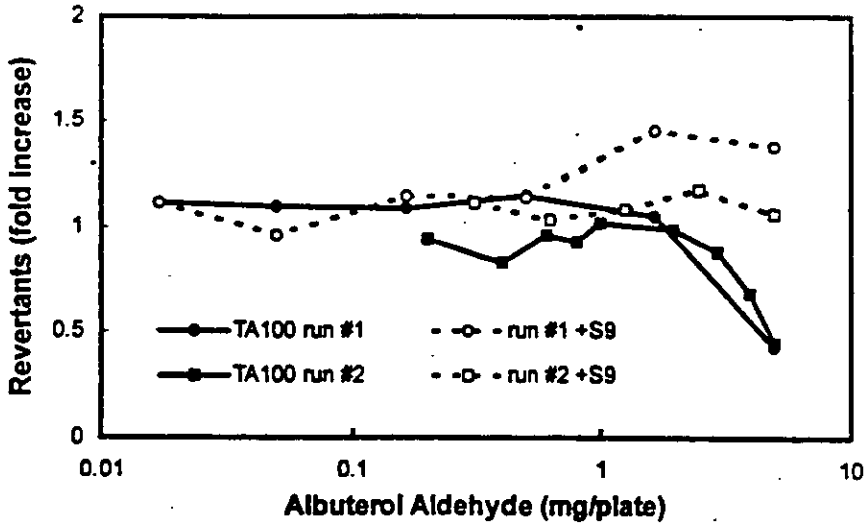
Figure 2. Mutagenic activity of ortho-hydroxy-ipratropium

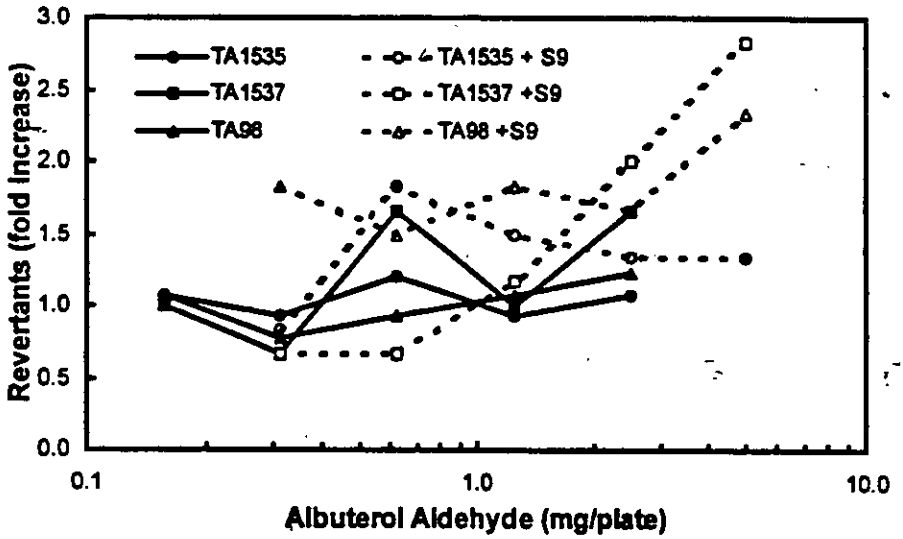
genic product but the parent compound could still exert a mutagenic effect. The extent of metabolism by induced microsomes *in vitro* does not necessarily predict the extent of metabolism *in vivo*. In conclusion, .meta- and para-hydroxy ipratropium were not mutagenic under the test conditions. Ortho-hydroxy-ipratropium, was weakly mutagenic at 2-5 mg/plate in the E. coli WP2 strain without metabolic activation.

Albuterol Derivatives: Hydroxy-albuterol was tested, with and without S9, at 0.313, 0.625, 1.25, 2.5, and 5.0 mg/plate. Toxicity was evident as markedly decreased colony number at the top dose in several strains. Without metabolic activation, toxicity occurred in all strains except TA100; with metabolic activation, toxicity occurred in strains TA1535 and TA1537. Hydroxy-albuterol did not increase revertant colony number under any of the test conditions. The test procedures were appropriate. Thus, hydroxy-albuterol was not mutagenic under the conditions of this test.

Results with albuterol aldehyde are summarized in figure 3, page 9. Preliminary cytotoxicity studies were done with the TA100 strain. Without metabolic activation, colony numbers were sharply decreased at 4-5 mg/plate. With metabolic activation, there was no effect on colony number. Based on the results with the TA100 strain, albuterol aldehyde was tested in the other strains at 0.16, 0.3, 0.6, 1.2, and 2.5 mg/plate without S9, and at 0.3, 0.6, 1.2, 2.5, and 5.0 mg/plate with S9. Without S9, there was no substantial increase in revertant colony number up to 2.5 mg/plate. In two of the strains (TA1537 and WP2), revertant colony number was increasing from 1.5 to 2.5 mg/plate; the compound was not tested at 5 mg/plate. In the presence of metabolic activation with S9, revertant colony number tended to increase dose-dependently in three strains (TA98, TA1537, and WP2). In strain TA 98 with S9, revertants increased 2.3-fold from [redacted] Absolute colony number was not greater than the maximum historical control value of 20 in any of the three replicate plates. The mean historical control is 12 (SD=4, N=96). Thus, despite the trend for increasing colony number, the test should be considered negative in this strain. In strain TA1537 with S9, revertant number increased 2.8 fold from a control value of [redacted] at 5 mg/plate. Absolute colony number in each of the three replicate plates (19, 15, and 16) was less than 20 but greater than the maximum historical control value of 10 (positive control = 39 at 2 μg/plate). The sponsor did not run a second experiment to determine whether this equivocal response is reproducible. In strain WP2 revertant number increased 2-fold from [redacted] at 5000 μg/plate (positive control = 149 at 2 μg/plate). In a second run revertant number increased 2.5-fold from [redacted] (positive control = 110 at 2 μg/plate and 501 at 4 μg/plate). The mean historical control is 60 (SD=22, N=55, MAX=125). Although neither run met the criterion of a 3-fold increase, the sponsor considered this a weak positive response based on a reproducible dose-related increase that, in one run, exceed the maximum historical control. Thus, albuterol aldehyde was considered mutagenic under conditions of metabolic activation in strain WP2, which detects base-pair substitutions, and elicited an equivocal response (not reproduced) under conditions of metabolic activation in strain TA1537, which detects frame shift mutations.

Bacterial Mutagenesis Assays





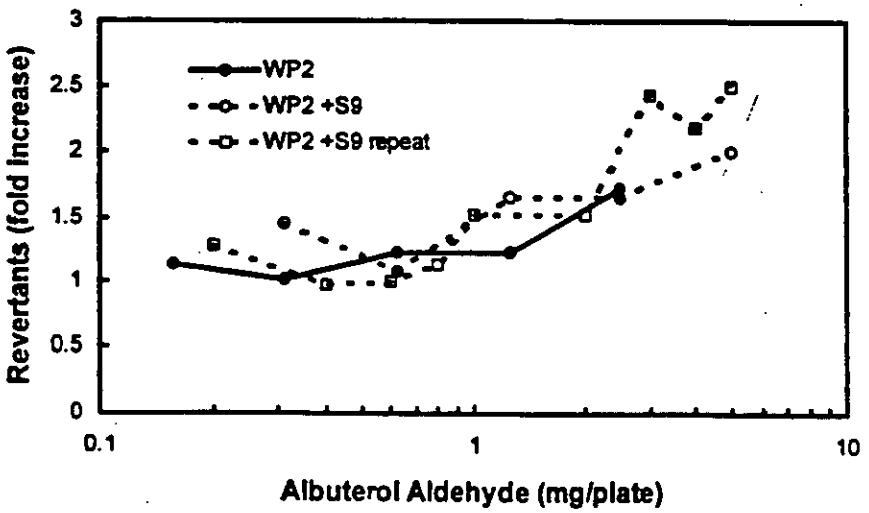
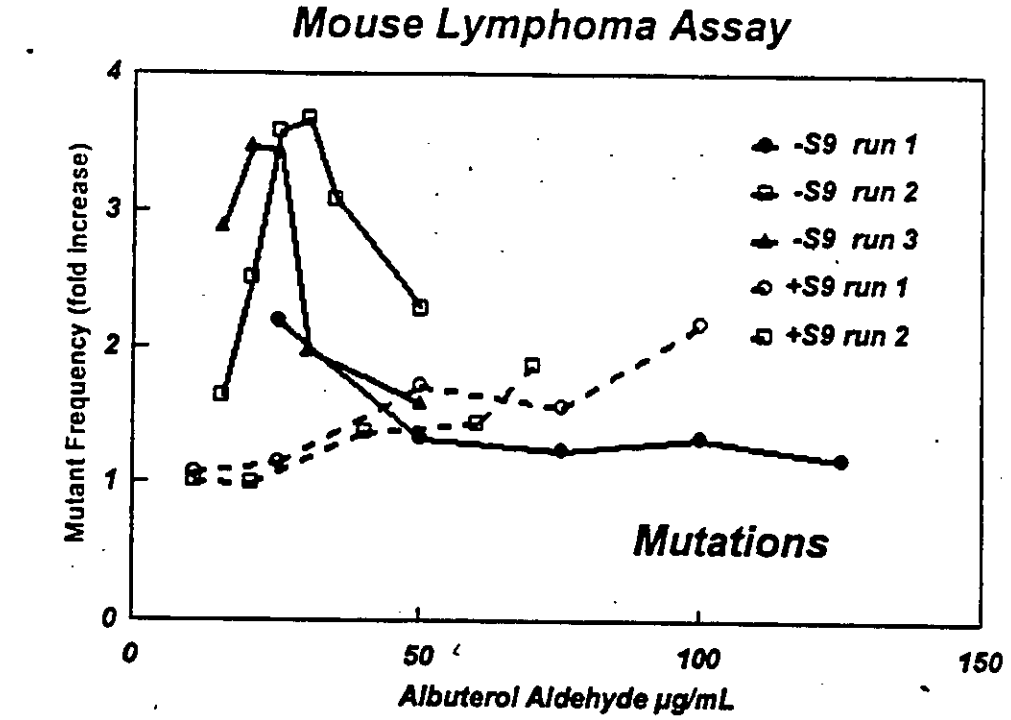
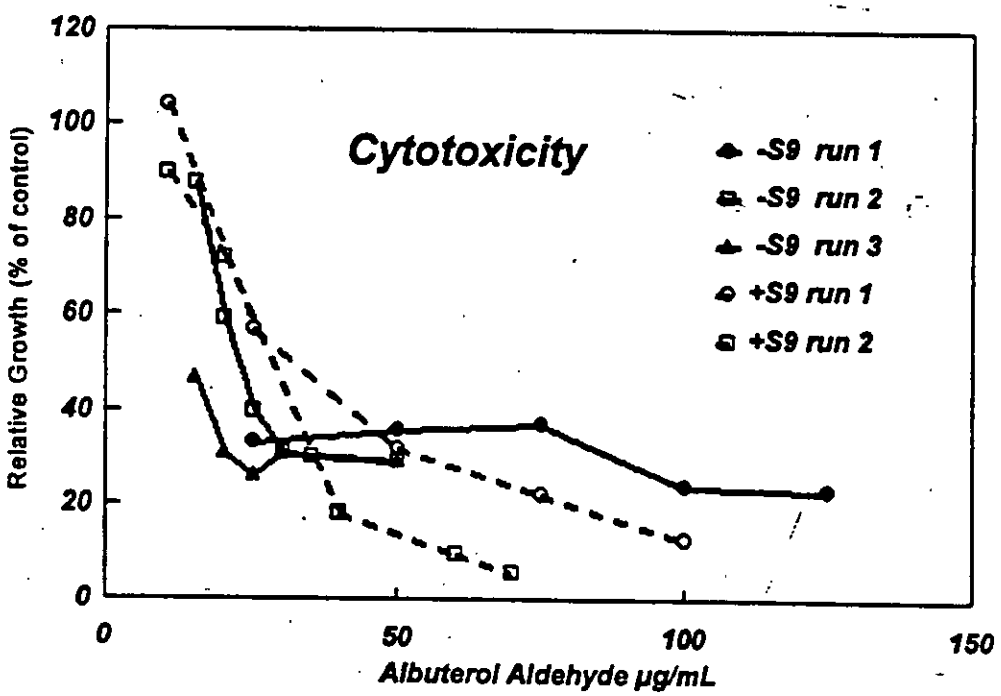


Figure 4


Mouse Lymphoma Assay
Mutant Frequency (fold Increase)
0
1
2
3
4
0
50
100
150
Albuterol Aldehyde µg/mL
-S9 run 1
-S9 run 2
-S9 run 3
+S9 run 1
+S9 run 2
Mutations


Relative Growth (% of control)
0
20
40
60
80
100
120
0
50
100
150
Albuterol Aldehyde µg/mL
Cytotoxicity
-S9 run 1
-S9 run 2
-S9 run 3
+S9 run 1
+S9 run 2

Comment: Hayes textbook of toxicology (third edition), specifically provides for a bell shaped dose-response in the mouse lymphoma assay. The data in the present case meet this criterion for a positive response. The text states:

> "An increase in mutant frequency may be followed by only small or no further increases at higher concentrations or toxicities. However, a decrease in mutant frequency to values below the minimum criterion is not acceptable in a single assay to classify the test article as a mutagen. If the mutagenic activity at lower concentrations or toxicities is large, a repeat assay is performed to confirm the mutagenic activity."

Albuterol Aldehyde: In Vitro CHO/HGPRT Mammalian Mutation Assay
Boehringer Ingelheim report # U95-3121, 19 JUN 95, vol 1, pg 379

Study Dates: 27 FEB 95 to 10 APR 95
Testing Lab: Boehringer Ingelheim Pharmaceuticals, Department of Toxicology and Safety Assessment, Ridgefield, CN
Test Article: Albuterol aldehyde (BIRG 597 BS), lot # 2439-98-1 batch E/94, [redacted]
GLP: The study was accompanied by a signed GLP statement.

Methods: Albuterol aldehyde was tested in the CHO/HGPRT assay with or without metabolic activation with liver S9 fraction from Aroclor-1254-induced rats. The CHO cells were adapted for suspension growth. No independent range finding assay was done. Doses were selected based on those used in the mouse lymphoma assay. Albuterol aldehyde was tested at 20 to 200 μg/mL without S9 and 20 to 70 μg/mL with S9. The high doses was targeted to decrease relative growth to ~20%. DMSO was the solvent control, ethyl methane sulfonate (without S9) and 3-methylcholanthrine were the positive controls. Duplicate cultures were exposed to test article or control treatment for 4 hours. After 9-10 days growth in suspension, cells were cloned in soft agar in the presence of 6-thioguanine to select for $HGPRT^-$ mutants and in non-selective medium to determine the cloning efficiency. After 10-12 days incubation colonies were counted with an automatic counter. Positive responses were defined as reproducible dose-related increases in mutant frequency greater than 3 times the average vehicle mutant frequency. The criteria could be modulate by other biological factors such as historical control data.

Results: In three runs without metabolic activation albuterol aldehyde was tested up to 50, 70, and 200 μg/mL with decreases in total relative growth to 47, 44, and 14% of control, respectively. In two runs with metabolic activation in the presence of S9 the compound was tested up to 70 μg/mL with a maximum decrease in total relative growth to 14%. There was no significant increase in mutant frequency under either set of test conditions. Positive controls gave expected responses. Thus, albuterol aldehyde was not mutagenic under these test conditions.

Albuterol Aldehyde: Mouse Micronucleus Assay
Boehringer Ingelheim report # U95-3124, 12 JUL 95, pg 457

Study Dates: 28 MAR 95 to 31 MAR 95
Testing Lab: Boehringer Ingelheim Pharmaceuticals, Department of Toxicology and Safety Assessment, Ridgefield, CN
Test Article: Albuterol aldehyde (BIRG 597 BS), lot # 2439-98-1 batch E/94, [redacted]
GLP: The study was accompanied by a signed GLP statement.

Methods: No independent range finding assay was done. The high dose (250 mg/kg/day for 3 days) was an estimate of the MTD, based on the acute toxicity study (Boehringer report # U95-3057, pg 268). Mice [CRL:CD1 (ICRBR) VAF$^+$ strain] were allotted to five groups (5/sex/group) that received vehicle (1% carboxymethyl cellulose/ 0.2% Tween 80), 63, 125, or 250 mg/kg/day albuterol aldehyde, or 1 mg/kg/day mitomycin C. Treatment was for 3 days; albuterol aldehyde and its vehicle were given by oral gavage, mitomycin C was given by i.p. injection. At ~24 hours after the last dose bone marrow slides were prepared from femurs. Coded slides were analyzed for the frequency of polychromatic (PCE) and normochromatic (NCE) erythrocytes. A decrease in the ratio of PCE/NCE in 200 erythrocytes was the measure of bone marrow toxicity. The incidence of multi-nucleated polychromatic erythrocytes (MN-PCE) was determined from 2000 PE examined per animal (except for one vehicle control animal in which 1400 were analyzed). Specific criteria for a positive response were not defined. Evaluation of the data was based on: "overall considerations including, where appropriate, the magnitude of response, reproducibility, dose-response relationships, interanimal variability, results in positive and negative control animals and groups and comparison of experimental results with historical data." Statistical tests were not considered necessary.

Results: One female treated with 125 mg/kg/day albuterol aldehyde was found dead on day 3 the cause of death was not determined. There were no other treatment related deaths; clinical signs of toxicity were not reported. There were no apparent differences among groups in the ratio of PCE/NCE (66% in vehicle controls). There were no apparent differences among vehicle and albuterol aldehyde groups in the incidence of MN-PCEs (0.14% in vehicle controls). The positive control increased the incidence of MN-PCEs 23-fold in males and 11-fold in females. The sponsor concludes that albuterol aldehyde did not increase the incidence of micronuclei under the conditions of this test.

Comment: The potential for albuterol aldehyde to produce micronuclei may not have been fully explored in this study. No toxicity was reported at the high dose of 250 mg/kg/day. Death occurred in one female in the mid-dose group (125 mg/kg/day) but since mortality was not dose-related it is not clear that the death was due to drug treatment. A previous acute toxicity study was used to support dose selection in the present micronucleus study. In that previous study mortality rates after single oral doses of 1000, 2500, and 5000 mg/kg were 0/5, 5/5 and 5/5, respectively in males, and 1/5, 5/5, and 5/5 in females.

Albuterol Aldehyde: Mouse Lymphoma *In Vitro* Mutation Assay
Boehringer Ingelheim report # U95-3119, 19 JUN 95, vol 1, pg 304

Study Dates: 31 JAN 95 to 10 MAR 95
Testing Lab: Boehringer Ingelheim Pharmaceuticals, Department of Toxicology and Safety Assessment, Ridgefield, CN
Test Article: Albuterol aldehyde (BIRG 597 BS), lot # 2439-98-1 batch E/94, [redacted]

GLP: The study was accompanied by a signed GLP statement.

Methods: Albuterol aldehyde was tested in the mouse lymphoma L5178Y $tk^{+/-}$ assay with or without metabolic activation with liver S9 fraction from Aroclor-1254-induced rats. Test concentrations were determined in preliminary cytotoxicity studies. Albuterol aldehyde was tested at 15 to 125 μg/mL without S9 and 10 to 100 μg/mL with S9. The high doses was targeted to decrease relative growth to ~20%. DMSO was the solvent control, ethyl methane sulfonate (without S9) and 3-methylcholanthrine were the positive controls. Two tubes of cells per concentration of test article or control were exposed to treatment for 4 hours. After a total of 48-hr, cells were cloned in the presence of trifluorothymidine to select for TK^- mutants and in non-selective medium to determine the cloning efficiency. After 10-12 days incubation colonies were counted with an automatic counter; the report does not specify whether large and small colonies were discriminated. Positive responses were defined as reproducible dose-related increases in mutant frequency greater than 2.5 times the average vehicle mutant frequency. The criteria could be modulated by other biological factors such as historical control data.

Results: Effects are summarized in figure 4, page 12. Two runs were conducted with metabolic activation in the presence of S9. Total relative growth decreased to 13% and 10% of control at 100 μg/mL and 60 μg/mL, respectively, in the two runs. There was no increase in mutation frequency in the absence of S9. Three runs were conducted without metabolic activation. In the first run, total relative growth decreased to 23% of control at 125 μg/mL. At the low dose of 25 μg/mL only, there was a 2.2-fold increase of mutation frequency. Consequently the test was repeated in two more runs at lower doses of 15 to 50 μg/mL. These runs revealed a "bell shaped" dose-response, with mutation frequency first increasing, then returning to baseline at higher doses. Mutation frequency peaked at 3.7- and 3.5-fold at concentrations of 30 and 20 μg/mL, respectively, in the two runs. The peak increases of mutant frequency were associated with decreases in total relative growth to 30-40% of control. The sponsor considered this to meet the criterion for a reproducible dose-related increase in mutant frequency. Thus, albuterol aldehyde was mutagenic under the conditions of this test.

ADDITIONAL GENOTOXICITY TESTS WITH ALBUTEROL ALDEHYDE:

Albterol Aldehyde: Human Lymphocyte *In Vitro* Cytogenetics Assay
Boehringer Ingelheim report # U95-3120, 19 JUN 95, pg 337

Study Dates: 21 FEB 95 to 18 APR 95
Testing Lab: Boehringer Ingelheim Pharmaceuticals, Department of Toxicology and Safety Assessment, Ridgefield, CN
Test Article: Albuterol aldehyde (BIRG 597 BS), lot # 2439-98-1 batch E/94, [redacted]

GLP: The study was accompanied by a signed GLP statement.

Methods: Albuterol aldehyde was tested in human peripheral blood lymphocytes with and without metabolic activation with liver S9 fraction from Aroclor-1254-induced rats. Test concentrations and positive controls were run in duplicate. DMSO was the negative control, mitomycin C (without S9) and cyclophosphamide (with S9) were the positive controls. Lymphocytes were treated for 48 hr with phytohemagglutinin to stimulate proliferation *in vitro*. Growth medium included 5% fetal bovine serum; serum was omitted in assays with metabolic activation. Without metabolic activation, cells were exposed to test article or control continuously for 24 hours ([redacted] μg/mL); with metabolic activation, cells were exposed to test article or control for 3 hr ([redacted] μg/mL). After a total of 70 hours, cells were treated with Colcemid® and 200 metaphase cells per dose (100 per culture) were analyzed microscopically. To calculate mitotic index, 500 cells/culture were scored for the presence of mitotic figures. Four categories of chromosome aberrations were reported; the incidence of gaps and polyploidy were recorded but not reported. The doubling time for these cells was not specified. A positive response was defined as a statistically significant (Chi²), dose-related, reproducible increase in aberration frequency over the concurrent negative control.

Results: Without metabolic activation, concentrations of albuterol aldehyde ≥ 15 μg/mL reduced mitotic index to < 35%, therefore, concentrations of 1, 5, and 10 μg/mL were scored for aberrations. Mitotic index decreased to 50% of negative control at 10 μg/mL. There was no significant increase of aberration frequency due to treatment with albuterol aldehyde. No aberrations were observed in negative controls; mitomycin C (0.5 μg/mL) increased the incidence of aberrations to 26%. With metabolic activation; albuterol aldehyde was tested at 50 to 80 μg/mL. Minimal cytotoxicity and no increase in aberrations was observed in this range. In a repeat test, mitotic index decreased to 46%, 57%, and 56% of negative control, respectively, at 120, 140, and 160 μg/mL. There was no significant increase in frequency of aberrations. Cyclophosphamide (3.5 μg/mL) increased the incidence of aberrations to 18%. This was an adequate test. The negative result without metabolic activation was not repeated, but it was performed with a relatively long exposure (24 hr) which would normally be a "second tier" condition for an initial negative response. Under the conditions of this test albuterol aldehyde was not clastogenic in human lymphocytes.

The median lethal dose for acute oral administration was, thus, between 1000 and 2500 mg/kg. The high dose for the micronucleus test should be a maximum tolerated dose (MTD) or 50% of the median lethal dose. It is not obvious that the dose of 250 mg/kg/day meets either of these criteria. Thus, the negative result claimed for this study must be tempered by the concern that a higher dose may have produced a positive result.

STUDIES WITH STABILITY BATCHES OF COMBIVENT UDV DRUG PRODUCT:

Bacterial (Ames) and mammalian (mouse lymphoma) in vitro mutagenesis tests were performed with a stability lot of Combivent UDV drug product. This material had been stored for more than 2 years and had an albuterol aldehyde content of 1.7 μg/mL, 0.14% relative to albuterol.

Combivent UDV Solution: Ames Bacterial Mutation Assay
Boehringer Ingelheim Report U95-3122, 06 JUL 95, pg 407

Study Dates: 18 APR 95 to 20 APR 95
Testing Lab: Boehringer Ingelheim Pharmaceuticals, Department of Toxicology and Safety Assessment, Ridgefield, CN
Test Article: Combivent UDV solution lot DE649 Analysis: [redacted] mg/mL albuterol sulfate, [redacted] mg/mL ipratropium bromide, [redacted] μg/mL albuterol aldehyde.
GLP: The study was accompanied by a signed GLP statement.

Methods: The test article was studied, with and without metabolic activation with liver S9 fraction from Aroclor-1254-induced rats. The [redacted] method was used with *Salmonella typhimurium* strains TA1535, TA1537, TA98, TA100, and *Escherichia coli* WP2 uvrA (pKM101). The bacterial strains' genetic phenotypes were verified by appropriate tests. No range finding was done. Doses tested were 0.16, 0.31, 0.63, 1.25 and 2.5 mL Combivent solution per plate. The maximum dose represents one Combivent vial and was the largest volume that could be accommodated in the assay. The upper dose corresponds to 3 mg/plate albuterol sulfate, 425 μg/plate ipratropium bromide, and 4.25 μg/plate albuterol aldehyde; ipratropium decomposition products were not assayed. Triplicate plates at each concentration were incubated for 48 hours. Colonies were counted manually or with an automated counter. The guidelines for a positive response were a reproducible, dose-related increase in mean revertant numbers. For strain TA100, which has a high baseline revertant mutation rate, an increase in mean revertant numbers to 2 times the solvent control was considered positive; for the other strains the criteria were a 3-fold increase over solvent control or an absolute number ≥ 20, whichever was greater. The solvent control was 0.88% NaCl, pH 3.4. Positive controls for the various strains with and without metabolic activation by liver S9 fraction were as follows:

Strain	Positive Controls	
	Without S9	With S9
TA1535	sodium azide 10 μg/plate	2-aminoanthracene 1 μg/plate
TA1537	9-aminoacridine 50 μg/plate	2-aminoanthracene 2 μg/plate
TA98	2-nitrofluorene 1 μg/plate	2-aminoanthracene 1 μg/plate
TA100	sodium azide 10 μg/plate	2-aminoanthracene 1 μg/plate
WP2 uvrA pKM101	N-ethyl-N'-nitro-N-nitrosoguanidine 0.5 μg/plate	2-aminoanthracene 2 μg/plate

Results: The test article had no cytotoxicity or mutagenicity under these conditions. The positive controls caused mutations as expected.

Comment: The sponsor concludes that these results support the genetic safety of Combivent UDV solution that has been stored for a minimum of 2 years. It is difficult to see what additional assurance this study provides over tests of the individual albuterol and ipratropium breakdown products. The final concentration of the decomposition products and even the parent drugs was too low to constitute an adequate test. It must be emphasized that the purpose of this assay is identification of mutagenic potential and not risk assessment. At best, this study shows that accumulation of breakdown products during storage does not result in marked synergy among the various components in producing mutations.

Combivent UDV Solution: Mouse Lymphoma Mutation Assay
Boehringer Ingelheim Report U95-3123, 06 JUL 95, pg 431

Study Dates: 18 APR 95 to 01 MAY 95
Testing Lab: Boehringer Ingelheim Pharmaceuticals, Department of Toxicology and Safety Assessment, Ridgefield, CN
Test Article: Combivent UDV solution lot DE649 Analysis: [redacted] mg/mL albuterol sulfate, [redacted] mg/mL ipratropium bromide, [redacted] μg/mL albuterol aldehyde.
GLP: The study was accompanied by a signed GLP statement.

Methods: The test article was examined in the mouse lymphoma L5178Y $tk^{+/-}$ assay with or without metabolic activation with liver S9 fraction from Aroclor-1254-induced rats. The maximum dose of Combivent solution was 0.6 mL/mL; this was the largest volume that could be accommodated in the assay. The upper dose corresponds to [redacted] mg/mL albuterol sulfate, [redacted] mg/mL ipratropium bromide, and [redacted] μg/mL albuterol aldehyde; ipratropium decomposition products were not assayed. The vehicle control was 0.88% NaCl, pH 3.4; ethyl methane sulfonate (without S9) and 3-methylcholanthrine were the positive controls. Two tubes of cells per concentration of test article or control were exposed to treatment for 4 hours. After a total of 48-hr, cells were cloned in the presence

of ipratropium bromide is taken as 100%, the potencies of the ortho- meta- and para-hydroxy analogs were 12.5%, 5.0%, and 1.8%, respectively (Boehringer report U95-3008, submitted to IND as serial 042, 13 FEB 95). The chronic toxicity of ipratropium is related, primarily, to its peripheral muscarinic blocking activity (e.g. dry nose, eye, and mucosa in dog and dry mucosa plus gastrointestinal and urinary dilation in rats, see pharmacology/toxicology review of NDA 20-228, 03 APR 92, C.J. Sun). Due to its quaternary ammonium structure, ipratropium has relatively low systemic availability. Pharmacokinetic data have not been submitted for the breakdown products but they share the quaternary ammonium group and it is likely that they also have low systemic availability. Each Combivent vial contains mg ipratropium. The proposed specification for each of the hydroxy-ipratropium compounds is <%; this would be µg each/day µg, q.i.d.) or µg/kg/day in a 50 kg patient. This is below the threshold level for biological qualification of impurities recommended in a recent step-2 draft ICH guideline on Impurities in New Drug Products (FR 3/19/96). The data presented do not raise any concern over acute toxicity of the breakdown products. These acute studies do not address potential chronic toxicity of these compounds. Because these breakdown products would be delivered in a relatively low dose, and because they lack the muscarinic blocking activity responsible for most of the chronic toxicity of ipratropium, further characterization of their chronic toxicity is not warranted at this time.

Albuterol Derivatives: Hydroxy-albuterol and albuterol aldehyde were each tested at 1000, 2500, and 5000 mg/kg, orally. The acute toxicity of albuterol aldehyde (LD_{50} < 2500 mg/kg) was greater than that of hydroxy-albuterol (LD50 ≥ 5000 mg/kg).

This observed mortality was in the range of that published in the literature for albuterol (LD_{50} = 1900-4700 mg/kg). The target of acute toxicity for both albuterol derivatives was the gastrointestinal tract, with red discoloration of the stomach mucosa. No data have been submitted concerning the β-adrenergic activity of the two albuterol derivatives, but, because of the alterations to the catechol ring, it is likely that they lack significant β-agonist activity. Relative to albuterol, the proposed specifications are ≤ % for hydroxy-albuterol (µg/day, µg/kg/day) and ≤ % for albuterol aldehyde (µg/day, µg/kg/day). As for the ipratropium breakdown products, these studies do not address chronic toxicity but do not raise any concerns for acute toxicity. The proposed levels are also below the threshold for qualification recommended in the draft ICH guideline. In the absence of any specific safety concerns, further characterization of the chronic toxicity of these compounds is not warranted at this time.

Genetic Toxicology:

Of the three ipratropium breakdown products only ortho-hydroxy-ipratropium exhibited genetic toxicity (in the E. coli WP2 uvA strain without metabolic activation). The sponsor's characterization of ortho-hydroxy-ipratropium as a "weak" mutagen is appropriate. The sponsor argues that the absence of mutagenic activity in the presence of metabolic activation suggests that the compound poses no genetic hazard *in vivo*. That argument is unacceptable. Ortho-hydroxy-ipratropium was not tested in a mammalian cell system. It is possible that this positively charged compound would have less access to

mammalian cells than to the bacterial tester strains that are genetically modified to increase cell permeability.

Of the two albuterol breakdown products only albuterol aldehyde exhibited genetic toxicity (in Salmonella strain TA1537 and E. coli strain WP2 uvA in the presence of. metabolic activation). Based on this weak mutagenic activity further tests were run. In the mouse lymphoma test albuterol aldehyde caused a "bell-shaped" increase in mutation frequency only in the absence of metabolic activation. Albuterol aldehyde was negative in the mammalian CHO/HGPRT mutagenicity test and in a test for chromosome aberrations in human lymphocytes. Albuterol aldehyde was negative in the mouse micronucleus test but it is not clear that a maximally tolerated dose was reached. Thus, albuterol aldehyde was weakly positive in 2/5 genetic toxicity screening tests, with no evidence of clastogenicity. It is not clear that additional tests would be useful in characterizing this activity.

Combivent UDV drug product on stability testing for over two years and with measurable albuterol aldehyde content was tested in bacterial and mouse lymphoma mutagenicity tests. These tests were negative but the interpretation is not obvious. Because of limitations of the concentrations available in the solution dose form, none of the constituents was tested up to a dose that would normally be considered adequate. There is precedent for a very highly mutagenic contaminant contributing to the apparent mutagenicity of a drug substance (Kropko et al, Mutation Res 281:233, 1992) but this is not the situation with the weakly mutagenic albuterol and ipratropium degradants.

The mutagenic potential of ortho-hydroxy ipratropium and albuterol aldehyde, although weak, would favor keeping the limits of these impurities as low as practicable. The specifications for the Combivent UDV solution marketed in the United Kingdom are < % for each of the three hydroxy-ipratropium compounds, ≤ % for hydroxy albuterol, and ≤ % for albuterol aldehyde. As background for a pre-NDA meeting on 29 JUL 96, the sponsor sent recent stability data to the Division by FAX dated 25 JUL 96. Stability data for 12 months at 25°C and 30°C are shown on pages 20 and 21. From these preliminary data it seems that it will be practical to limit albuterol aldehyde and ortho-hydroxy ipratropium (BIRJ 0420) to less than % and possibly below %. An upper limit of % would be a total daily intake of μg albuterol aldehyde and μg of ortho-hydroxy ipratropium.

APPEARS THIS WAY
ON ORIGINAL

COMBIVENT® Inhalation Solution
Stability Summary

Manufacturing Site: Boehringer Ingelheim, Ltd., UK
Manufacture Date: November 1994
Package Configurations: #1, LDPE Vials packed in carton boxes
#2, LDPE Vials packed in aluminum foils
Storage Conditions: 12 months at 25°C/60%RH

Package	Mfr. Batch No.	Volume of Contents Vt/Vi (ml/vial)	Assay IPB At/Ai (mg/2.5ml)	Assay ABS At/Ai (mg/2.5ml)	Albuterol Aldehyde %	Hydroxy Albuterol %	Tropic Acid %	BIRJ 0420 %	BIRJ 0416 %	BIRT 0109 %	BA 686 %
#1 Box	DE754										
#2 Foil	DE754										
#1 Box	DE755										
#2 Foil	DE755										
#1 Box	DE756										
#2 Foil	DE756										

(1) ND = < LOD = 0.01 %

Vi = initial volume Vt = volume at time (t) Ai = initial assay At = assay at time (t)

NOTE: Values for impurities / decomposition products are reported as % decomposition of the active ingredient

IND
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COMBIVENT® Inhalation Solution
Stability Summary

Manufacturing Site: Boehringer Ingelheim, Ltd., UK
Manufacture Date: November 1994
Package Configurations: #1, LDPE Vials packed in carton boxes
#2, LDPE Vials packed in aluminum foils

Storage Conditions: 12 months at 30°C/70%RH

Package	Mfr. Batch No.	Volume of Contents Vt/Vi (ml/vial)	Assay IPB At/Ai (mg/2.5ml)	Assay ABS At/Ai (mg/2.5ml)	Albuterol Aldehyde %	Hydroxy Albuterol %	Tropic Acid %	BIRJ 0420 %	BIRJ 0416 %	BIRT 0109 %	BA 696 %
#1 Box	DE754										
#2 Foil	DE754										
#1 Box	DE755										
#2 Foil	DE755										
#1 Box	DE756										
#2 Foil	DE756										

Vi = initial volume Vt = volume at time (t) Ai = initial assay At = assay at time (t)

NOTE: Values for impurities / decomposition products are reported as % decomposition of the active ingredient

RECOMMENDATION

Two breakdown products in Combivent UDV solution have been identified with weak mutagenic activity. Combivent would be indicated for a severe pulmonary disease in adults. A prudent course would be to allow development of the Combivent UDV inhalation solution but to keep the specifications on impurities as low as practicable. Specific limits should be recommended during the NDA review in consultation with the reviewing chemist based on stability data not yet available. Preliminary data suggest that it may be practical to limit both impurities to less than % or %. Because of the mutagenic potential of these degradants, a limit of < would be preferable; if a limit of < % is not practicable from a chemistry standpoint, then, a limit of < % would be acceptable. Additional measures to inhibit degradation, such as refrigeration and overwrapping, should be investigated. Specifications for albuterol degradation in other solution formulations may need to be re-evaluated in light of the mutagenic potential of albuterol aldehyde.

Mark Vogel, Ph.D., Pharmacologist

Original IND
c.c. HFD-570/Division File
HFD-570/C.J. Sun
HFD-570/B. Otulana
HFD-570/P. Jani
HFD-570/W.M. Vogel
HFD-570/J. Leak
HFD-570/G. Poochikian

APPEARS THIS WAY ON ORIGINAL

MAY 12 1998

Division of Pulmonary Drug Products

Review of Pharmacology/Toxicology Data

Original Review

Reviewer : VEWhitehurst

Review Completion Date : February 20, 1998.

Information to be Conveyed to the Sponsor : Yes

HFD : HFD 570

NDA : NDA 20-837

Sponsor : Sepracor
111 Locke Drive
Marlborough, MA 01752

Drug : [redacted](Levalbuterol HCL) Inhalation Solution

Category : Beta agonist

Indication : Treatment or prevention of [redacted]bronchospasm in patients [redacted] years of age and older with reversible obstructive airway disease and [redacted]

Administration : Inhalation

Composition and Dosage Forms :

Components	**1.25 mg (mg/ 3ml)**	**0.63 mg (mg/3ml)**
[redacted]		

Levalbuterol HCL (mg)
Sodium chloride
Sulfuric acid,

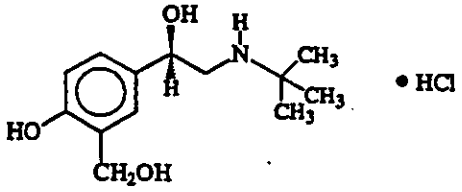
USAN : Levalbuterol Hydrochloride

Chemical name : (R)-α1-[[(1,1-dimethylethyl(amino]methyl]-4-hydroxy-1,3benezenedimethaniol hydrochloride

Molecular Weight : 275.8

Cas Registry # : 50293-90-8

Chemical Structure :



Related IND/NDA : IND

Proposed Clinical Dose : In patients 12 years and older, the daily doses are 0.63 mg, or 1.25 mg inhaled 3 x daily,

Previous Clinical Experience : Racemic albuterol inhalational solution has been approved for the treatment of [redacted] bronchospasm in patients 12 years and older for more than 15 years in 30 countries worldwide. Racemic albuterol has been shown to be very effective in the treatment of bronchospasm in the asthma patient. In contrast with racemic albuterol which contains equal parts of R and S enantiomers, [redacted] is composed of the R enantiomer only. Data developed by the sponsor suggest that the R enantiomer is as potent and/or is slightly more potent than racemic albuterol while having the potential to be less toxic, i.e., less adverse effects on the cardiovascular and central nervous systems. The sponsor suggests that their data show that R- enantioner is responsible for beneficial aspects of this drug and the S-enantioner is responsible for most of the adverse events associated with racemic albuterol.

Disclaimer Note : Some of the materials in this review may be taken directly from the sponsor's submission.

Introduction and History : [redacted] was submitted as a 505 (b) (2) and can reply on data from original NDAs (i.e., NDA 18,473) for racemic albuterol. In a meeting in 1992, the FDA recommended that the sponsor carry out 28 day toxicity studies in a rodent and non-rodent species and then carry out a 90 day toxicity study in the most sensitive animal species. The objective of these studies was to confirm that the toxicological profile for [redacted] was similar to that of racemic albuterol. Additionally, FDA recommended that the sponsor carry out a teratology study to investigate the effects of R-albuterol on embryo-fetal development.

Index of Preclinical Studies :

Toxicology Studies
Acute Studies:
1. Single Dose Study in the Mouse (study # 889/003, March 30, 1994).
2. Single dose study in the Mouse (study # 899/004, July 7, 1994).

Subchronic Studies :

1. 7 Day Oral Toxicity Study in the Rat (study # 051-800, January 12, 1995).
2. 5 Day Dose Ranging Study in the Rat (study # 051-804, April 8, 1997).
3. 28 Day Oral Toxicity Study in the Rat (study # 051-801, February 28, 1995).
4. 28 Day Repeat Inhalation Toxicity Study in Rats (study # 051-804, February 28, 1995).
5. 90 Day Oral Toxicity study in the Rat (study # 051-802, January 29, 1995).
6. 90 Day Inhalation Toxicity Study in the Rat (study # 051-806, 1995).
7. Preliminary Inhalation Study in the Dog (study # 051-811, 1997).
8. 7 day Toxicity Study in the Dog (study # 051-815, 1997).
9. 7 day Dose-Ranging Toxicity Study in the Dog (study # 051-812,1997).
10. 90 day Inhalation Toxicity Study in the Dog (study # 051-816

Reproduction Studies :

1. Study for Effects on Embryo-Fetal Development in Rabbits (study # 051-807, March 12, 1997).

Genotoxicity Assays:

1.Ames Genotoxicity Study (study 051-804, January 15, 1996)
2.CHO/HRPT Mammalian Cell Forward Gene Mutation Assay (study # 0314FS23001, March 12, 1997).

Pharmacology Studies: :

Mechanism Of Action : [redacted] interacts with beta $_2$ receptors in the pulmonary tissues activating the enzyme, adenylate cyclase, stimulating intracellular cyclic AMP leading to several cellular responses, namely activation of protein kinase A, reduction of intracellular calcium concentration as well as reducing the sensitivity of the contractile proteins to calcium. These responses result in the relaxation of the smooth muscle cells leading to bronchodilation. [redacted] also has the potential to interact with beta $_1$ and beta $_2$ adrenergic receptors in the cardiovascular tissues activating cyclic AMP leading to tachycardia, myocardial necrosis and sudden death.

Drug Activity Leading to Proposed indication :

a. In vitro Studies:

1. Binding Properties of (R), (S) and R/S -Albuterol to Beta - Adrenergic Receptor Subtypes (study # 051-402, 1994).

R-, S - and racemic albuterol was evaluated in radioligand assays to determine respective affinities for $beta_1$ and $beta_2$ adrenergic subtypes. Each compound was tested at 10 different concentrations (10^{-4}-10^{-9}) in binding assays using rat heart ($beta_1$ adrenceptor) and guinea pig lung ($beta_2$ adrenceptor) Membrane preparations. IC_{50} values were determined by non-linear analyses.

Compound	**$Beta_1$ -Adrenceptor IC_{50} (μM)**	**$Beta_2$-Adrenceptor IC_{50} (μM)**
R-Albuterol	9.9	4.1
S-Albuterol	>100	>100
R/S Albuterol	15.6	5.2

A comparison of R -Albuterol IC_{50} values for $beta_1$and $beta_2$ adrenceptors indicates a 2.3 fold selectivity for the $beta_1$ adrenceptors. A similar ratio was found for racemic albuterol while S-albuterol seem to be inactive for receptors in this assay.

In this assay, the data suggest that R-albuterol had a greater affinity for $beta_1$ than for $beta_2$ receptors. The same conclusion was shown for racemic albuterol. S-albuterol had a low affinity for $beta_1$ and $beta_2$ adrenceptor receptors in these assays. These data would suggest that R- and racemic albuterol have a greater potential to induce toxicity in the cardiovascular system than to induce bronchodilation in the pulmonary tissues.

2. Effects of Salbutamol and Its Isomers on Antigen-Induced Histamine Release from Human leukocytes (study # 051-401,1993).

The effects of R-, S- salbutamol and racemic Salbutamol on antigen-induced histamine release were evaluated in an in vitro leukocyte assay. Human white blood cells were incubated with anti-IG_E and R-, S- and racemic salbutamol (10^{-4}- 10^{-9} M) for 60 minutes at 47°C. The cells were measured for histamine release using a [redacted]

Anti IG_E -Induced Histamine Release in Human Leukocytes.

Concentration (M)	**RS Salbutamol** % Release	**R- Salbutamol** % Release	**S- Salbutamol** % Release
10^{-9}	100.7	99.9	105.5
10^{-8}	95.0	93.1	99.1
10^{-7}	82.3	83.4	105.7
10^{-6}	88.1	78	100.1
10^{-5}	85.3	76.4	93
10^{-4}	80.1	77.9	87.6

These data suggest that R- and racemic salbutamol inhibit histamine release from human leukocytes. At a concentration of 10^{-7} M, R-salbutamol inhibited histamine release by approximately 18 %. Similar results were obtained with racemic salbutamol while S-salbutamol had no inhibitory effects on histamine at this concentration. However, at a concentration of 10^{-4} M, R -albuterol inhibited histamine release by approximately 22 %, R/S albuterol inhibited histamine release by approximately 20 % and S-albuterol inhibited histamine release by approximately 12 %.

3.Studies in Coronary Hyperreactivity Effects of Beta- Adrenergic Drugs and Their Optically active Isomers (study # 051-453, 1993).

The aim of this study is to investigate the ability of racemic albuterol, R- and S-albuterol and its enantioners to induce relaxation and/or hyperreactivity in isolated bovine arteries. The bovine preparations were incubated with either R, S or R/S albuterol for 90 minutes (10^{-5}). The

preparations were then washed and contracted with increasing doses of histamine, carbachol or 5- hydroxytryptamine (5-HT).
R - and racemic albuterol were effective inducing relaxation in the bovine preparations contracted with histamine. R-and racemic albuterol were ineffective in inducing relaxation in the preparations contracted with carbachol or 5-HT. S- albuterol was ineffective in its ability to relax the preparations incubated with histamine, carbachol or 5-HT. Incubation with S-albuterol also increased the sensitivity to 5-HT.

4.Comparsion of Isoproterenol and R-, S- Albuterol and R/S Albuterol Binding to Human Beta$_2$-Adrenergic Receptors and Activities of Cyclic AMP Production (study # 050-400, 1992).

The affinity of R-, S-albuterol and R/S albuterol for recombinant human beta$_2$ adrenocepters were determined in binding assays. Each compound was evaluated at 9 concentrations (10^{-3} to 10^{-11}) in radioligand assays using

The time course of R-, S- and R/S albuterol to stimulate cyclic AMP using human prostate cells (PC3). Characterization of the PC3 cells demonstrate that each cell contained 21,000 beta adrenoceptors per cell of which 95 % were beta$_2$ adrenoceptor receptors. The results are listed below :

Compound	**Beta $_2$-Adreneroceptor K_d (nM)**	**cAMP Stimulation EC_{50} (nM)**
R-Albuterol	275	100
S-Albuterol	25000	14000
R/S Albuterol	400	207
Isoproterenol	32	12

K_d = dissociation constant
EC_{50} = maximum elevation in cAMP

R-Albuterol was 2 fold more potent in binding to beta$_2$ adrenoceptor receptors (275 to 400 nM) and stimulating cAMP than R/S Albuterol (100 to 207 nM) in this assay using [redacted] However, these compounds were less binding than isoproterenol by approximately 9 and 13 %, respectively. All 3 compounds rapidly stimulated cAMP (T1/2 = 2 minutes) followed by a plateau (10-60 minutes) and then a decline in cAMP even in the presence of a PDE inhibitor, IBMX. S-albuterol was much less potent at binding to beta adrenergic receptors and stimulating cAMP production.

6. Comparison of R-, S- and R/S-Albuterol and R/S Salmeterol Binding to Human Beta $_1$ and Beta $_2$-Adrenergic Receptors (study # 051-406,1994).

R-, S- R/S albuterol were evaluated in radioligand assays to determine their respectively affinities for beta$_1$ and beta $_2$ adrenceptor subtypes. Each agonist was evaluated at 7 concentrations (10^{-4}-10^{-11} M) in binding assays using [redacted] Results are show below :

Binding of Compounds to Beta$_1$ and Beta$_2$ adrenceptor subtypes

Compounds	**Beta $_1$ Adrenceptor (IC_{50} μM)**	**Beta $_2$ adrenceptor (IC_{50} μM)**
R-albuterol	1540	236
S-albuterol	111000	34000
R/S albuterol	2980	668

R- albuterol had approximately 3 and 125 x greater affinity for beta$_2$ adrenceptor receptors than racemic or S-albuterol. Additonally, R-albuterol had a 7 fold greater affinity for beta$_2$ adrenceptor than for beta$_1$ receptor.

7. Desensitization of the Human Beta$_2$-Adrenoceptor Receptors Induced by Isoproterenol and R-, S- and R/S Albuterol (050-401, 1993).

The ability of Isoproterenol, R-, S- and R/S albuterol to desensitize the $beta_2$ adrenoceptor receptors in human prostate cells (PC3) was investigated. PC3 cells were incubated with saturating concentrations of the agonists for periods of 5 minutes to 24 hours.
R -and racemic albuterol were very similar in loss of cAMP production and loss of beta adrenergic number, T1/2 = 90 and 160 minutes, respectively. Isoproterenol induced the most rapid loss of cAMP production, T1/2 = 10 minutes, loss of beta adrenergic, T1/2 =100 minutes. S-albuterol promoted much slower loss of cAMP production (T1/2 = 290 minutes) and loss in the numbers beta adrenergic receptors (300 minutes). This data suggest that S- albuterol has the potential to induce greater and more severe toxicities than R- and R/S albuterol. However, S-albuterol may have the potential to cause greater cardiovascular toxicity because the adrenergic receptors are available to interact with agonists stimulate cAMP leading to toxicity.

8. Long Term Desensitization of Human Beta $_2$-Adrenergic Receptors Induced by R-, S- and R/S Albuterol (study # 050-403, 1994).

The effects of R-, S- and R/S Albuterol and Isoproterenol on desensitization of $beta_2$ adrenoceptors were studied using human bronchial cells which have been characterized to contain approximately 8500 beta adrenoceptors per cell. The cells were incubated with the agonists for 48 hours.

The Effects of Several Compounds on Long Term $Beta_2$-Adrenoceptors Desensitization.

Pretreatment	Concentrations (μM)	cAMP Response	$Beta_2$ Adrenceptor Number
Isoproterenol	50	100	80
R/S Albuterol	100	98	80
	10	100	86
	1	74	91

	0.5	100	91
	0.1	94	88
	0.02	97	85
R-Albuterol	50	98	74
	5	98	88
	0.5	81	93
	0.25	95	89
	0.05	77	90
	0.01	80	82
S-Albuterol	50	99	82
	5	88	84
	0.5	**61**	**67**
	0.25	**44**	**50**
	0.05	**0**	**25**

The results of this assay show that these agonists desensitize $beta_2$ adrenoceptors. R albuterol at an 0.01 μM concentration induced an 82 % reduction in $beta_2$ adrenocepter number and a corresponding 80 % loss in cAMP response. Similar results were observed with racemic albuterol at a concentration of 0.02 μM, 97 % reduction in the number of $beta_2$ adrenoceptor receptor and a corresponding 80 % loss in cAMP production. S-albuterol was less potent, a 0.01 μM concentration had no effect on cAMP response (0% loss) and only a 7% loss in adrenceptor receptor loss. These data suggest that S-albuterol may be less deleterious to the bronchial cells than R-and R/S albuterol.

9. Effects of R-and S -Enantiomers of albuterol on Stimulated Secretion of Eosinophil Peroxidase in Human Eosinophils (study #051-423,1995).

The study examined the relative effects of R-, S-and R/S albuterol on the cellular activation of peripheral eosinophils from normal and atopic volunteers. Cellular activation was quantitated kinetically by the release of eosinophil peroxidase (EPO), a reliable and sensitive protein marker of

degranulation. R-, S- and racemic albuterol were incubated with eosinophils at concentrations of 10^{-10}, 10^{-4} and 10^{-6} M with incubation times of 5, 15 and 30 minutes. Results of this study show that the optimal time was 5 minutes. Both R- and racemic albuterol exhibited dose-related inhibition of the release of EPO in eosinophils from normal and atopic donors. S- albuterol augmented the release of EPO from eosinophils in this study.

10. **S-Albuterol is a Novel Activator of Phospholipase C in Airway Smooth Muscle. A Possible Link to the Asthma Paradox (study # 051-478, 1997).**

The objective of this study was to determine the effects of S-albuterol on intracellular free calcium concentration and inositol 1, 4, 5 triphosphate (IP_3) production in dissociated bovine tracheal smooth muscle. The smooth muscle was incubated with S-albuterol, 10μM for 30 seconds (n=4). S-albuterol induced a transient increase in calcium from [redacted] μM. S-albuterol also IP_3 by approximately 200 %. These data suggest that S-albuterol has the potential to increase intracellular calcium which results in a more forceful smooth muscle contraction. This effect may play a role in bronchial hyperresponsiveness/ hyperreactivity associated with racemic albuterol.

In Vivo studies :

1. **Effects of R/S Albuterol on Antigen- Induced Hyperreactivity in Guinea Pigs (study # 051-410, 1995).**

The object of this study was to calculate the effects of racemic albuterol on airway hyperreactivity in passively- sensitized male and female guinea pigs, 6/sex (Hartley strain/6/group). The guinea pigs were administered albuterol, I mg/kg daily, sc for 6 days. The animals were then instrumented for pulmonary measurement to evaluate the modulate airway hyperreactivity to histamine (1.5-10 μg/kg) or leukotriene C_4 (180-1500 ng/kg). The spasmogens were given iv. Following the spasmogen challenge, bronchial alveolar lavage was performed and cellular recruitment was evaluated, particularly the eosinophils.

The results of this study show that racemic albuterol did not antagonize airway hyperreactivity induced by histamine or leukotrienes. However, racemic albuterol did reduced the number of eosinophils in the bronchial alveolar lavage.

2. Effect of R-, S- and R/S Albuterol or S,S Formoterol on the Development of Antigen-Mediated Airway Hyperreactivity in the Guinea Pig (study # 051-443, 1996).

The objective of this study was to evaluate the effects of R-, S- and R/S Albuterol on airway hyperreactivity in passively sensitized male and female, Hartley strain guinea pigs (6/group). The methodology was the same as described above. The animals were given 1 mg/kg of sc daily for 6 days, instrumented for pulmonary airway measurement, then subjected to increasing doses of histamine (1.5-20 μg/kg) or leukotriene C_4 (1.5-6.0 μg/kg), iv. After the spasmogen challenge, bronchial alveolar lavage was carried out.
Results of this study reveal that racemic albuterol caused a significant increase in airway hyperreactivity in the sensitized guinea pig when leukotriene was used as the spasmogen. There was no change in airway responsiveness when the animals were treated with R-, or S-albuterol. Analyses of the lavage fluid of these animals show no significant reduction in eosinophils or macrophages.

3.Pulmonary Resistance Assay in Guinea Pigs (study # 050-402, 1993).

R-, S- and racemic albuterol were evaluated for the inhibition of bronchospasm induced by acetylcholine in anesthetized male Hartley guinea pigs (12). The animals were instrumented for pulmonary measurement, then given acetylcholine (2.5-15 μg/kg) to produce submaximal bronchoconstriction. 10 minutes after the spasmogen, the agonists were given to the guinea pigs using iv doses of 10, 30 and 100 mg/kg of R-, S- or R/S albuterol.

Pulmonary Function After Acetylcholine Treatment

Treatment (mg/kg)	Pulmonary Resistance	Intrapleural Pressure	Compliance
	(percent from baseline)		
Saline	-6.8	-13	+13
	+19	-6	+6
	-31	-45	+13
R/S*			
	-58	-43	+27
30	-67	-56	+28
100	-80	-69	+28
R-*			
10	-43	-31	+20
100	-86	-79	+18
S-*			
10	+43	+51	-15
100	+28	+7	-6

* = albuterol

IV administration of R- and R/S Albuterol produced dose-related decreases in pulmonary resistance and intrapleural pressure in guinea pigs induced by acetylcholine. S- Albuterol was ineffective in these studies.

4. Pulmonary Resistance Assay in the Guinea Pigs (study # 050-405, 1993).

The objective of this study was to evaluate the inhibition of histamine-induced bronchospasm in anesthetized guinea pig (Hartley strain, 6/dose group). R-, S- and racemic albuterol were administered IV, 100 mg/kg, the

animals were then instrumented for pulmonary measurements. The animals were then subjected to increases doses of IV histamine, 5-25 µg/kg.

Pulmonary Function After Histamine:

Treatment	Pulmonary Resistance (Change from Baseline)	Intrapleural Pressure	Compliance
Saline	+10	+48	+5
R/S-Albuterol	-67	-60	+45
R-albuterol	-65	-58	+68
S-Albuterol	unchanged	-7	+13

R-and R/S Albuterol decreased pulmonary and intrapleural pressure in guinea pigs induced by histamine. S-Albuterol had no effect on histamine-induced pulmonary resistance or intrapleural pressure.

Summary of the Pharmacology Studies :

Pharmacology studies include in vitro and in vivo studies to characterize effects on airway and non-airway tissues, beta adrenoceptor binding in animal and human tissues including the potential of R-Albuterol to stimulate cAMP elevation and to desensitize beta receptors. These studies show :

1. The binding potential of R- and racemic albuterol for beta adrenoceptor receptors were similar in membrane assays using rat heart and guinea pig lung tissues.
2. R-Albuterol was 2 fold more potent than racemic albuterol in binding to beta $_2$ adrenoceptor receptors and stimulating maximum cAMP elevations.
3. R-Albuterol and racemic albuterol attenuated the increase in pulmonary resistance in guinea pigs induced by spasmogens, i.e., histamine and acetylcholine.
4. Both R-Albuterol and racemic albuterol have the ability to desensitize beta adrenoceptor receptors (loss of beta adrenoceptors and loss of

cAMP production). However, R -Albuterol was less potent than racemic albuterol and isoproterenol in this effect on the adrenoceptor receptors.

Safety Pharmacology :

1.Effects of Albuterol on Locomotor Activity (study # 051-477, 1996).

The objective of this study was to evaluate the ability of the isomers of albuterol to stimulate or inhibit locomotor activity in mice. Doses used in this study were 3, 10, 30 and 199 mg/kg, IP. The results indicate that the isomers of albuterol did not change locomotor activity in mice.

2. Examination of 3 Compounds for tremorigenic Properties in Mice (study # 051-414, 1995).

The tremor-inducing effects of R-, S-and R/S albuterol was tested in mice in a mouse model of tremor. The mice (10/dose) were treated with L-DOPA, a catecholamine that primes the beta adrenergic system. The mice were treated orally with either R-, S-or R/S albuterol (32, 64 or 128 mg/kg) and observed for dose-related effects.

Tremorgenic Effects of Compounds

Treatment	**32 mg/kg**	**64 mg/kg**	**128 mg/kg**	**Vehicle**
R-Albuterol	9/10	7/10	10/10	2/10
S-Albuterol	10/10	5/10	10/10	2/10
R/S Albuterol	8/10	9/10	10/10	2/10

R-, S-and R/S albuterol induced sustained tremor at all doses used in this study. It is unclear whether there was a dose -related effect with racemic albuterol.

3. Evaluating the Comparative Effects of R/S Albuterol, R-Albuterol and S-Albuterol on Heart Rate, Blood Pressure and the Lead II EKG Following IV Administration to the Conscious Dog (study # 051-404).

The objective of this study is to compare the cardiovascular effects of R-, S- and R/S Albuterol in conscious dogs (n=6). The dogs were treated with these agonists using IV doses of 0.1 to 100 to 300 mg/kg. The following parameters were measured 30 minutes postdosing : heart rate, blood pressure,QRS interval, PR interval, QT interval and QT_C. In addition, blood was taken and evaluated for plasma potassium and glucose.
The results of this study indicate that IV administration of R- and racemic albuterol to conscious dogs produced dose-related increased in heart rate from 1 to 100 mg/kg. At an IV dose of 100 mg/kg, the increase in heart rate was approximately 140%. At this dose, there was a 10 % increase in QT_C.
At these doses , the blood pressure in these dogs were significantly decreased (35-50%). R-Albuterol, 100 mg/kg decreased plasma potassium from 3.6 to 2.5 mmol/L and increased plasma glucose from 79 to 161 mg/dl. Similar findings were found with racemic albuterol.

Summary of Safety Pharmacology :
There were limited safety pharmacology studies in this submission. The studies were to investigate the effect of albuterol on locomotor activity and tremor in the mouse and on the cardiovascular system in the dog. These studies reveal :

1. R- Albuterol had no effect on locomotor in the mouse.
2. R- Albuterol was tremorigenic in mice, the effect was dose-related.
3. R-Albuterol induced dose-related decreases in blood pressure and concurrent increases in heart rate in the dog. Additionally, the drug caused decreases in plasma potassium as well as increases in plasma glucose concentrations.
4. Similar findings were observed with racemic albuterol.

Preclinical Studies

Acute Toxicity using [redacted] **(R Albuterol Study # 899-003, Batch 002/146).**

Acute toxicity results are summarized below :

Drug	Dose (mg/kg)	Route	Mortality	Symptoms
	15	iv	0/4	lethargy, tremors
	30		0/4	lethargy, tremors
	60		1/4	lethargy, tremors
	120		4/4	lethargy, tremors
R/S albuterol	15	iv	0/4	Same symptoms
	30		0/4	as R-albuterol
	60		1/4	
	120		4/4	
S-albuterol	15	iv	0/4	Same symptoms
	30		0/4	R-albuterol
	60		0/4	
	120		4/4	

(Acute Study # 899-004)

Drug	Dose	Route	Mortality	Percent
R-albuterol	45	IV	0/10	0
(Lot # 22146)	60		3/10	30
	75		9/10	90
	90		8/10	80
R/S albuterol	45	IV	0/10	0
	60		2/10	20
	75		5/10	50
	90		10/10	100

S-Albuterol	45	iv	0/10	0
	60		1/10	10
	75		3/10	30
	90		510	50

Summary of the Acute Toxicity : LD_{50} for R-, S and R/S albuterol administered by the IV route was calculated to be approximately 66-70 mg/kg in male and female mice. Deaths occurred within 15 minutes after dosing and were thought to be related to cardiovascular or CNS accidents. Clinical signs include lethargy and tremors. There were no effects on body weight gain.

Subchronic Toxicity :

1. 7 day Oral Toxicity Study in the Rat (study # 051-800; Lot 33H0576; January 28, 1995).

GLP Study : No

Laboratory :

Animal Species/ Strain : Rat, Sprague-Dawley- Crl: CD (SD) BR

Number of Animals/Dose: 5/sex

Formulation : Racemic albuterol was dissolved in)% methylcellulose.

Route of Administration : Oral gavage

Frequency and Length of the Study: Drug given once daily/7 days.

Dose Design :

Group	Dose (mg/kg)

1	0 (vehicle)
2	1
3	25
4	50
5	100
6	200

Results :
Mortality : There were no deaths in this study.

Clinical Observations : There were no clinical signs observed for any of the animals in this study.

Body Weights: At study termination, body weight gain was found in the rats in the 25, 50, 100 and 200 mg/kg groups. The body weight gain was approximately 2-6 %.

Food Consumption : End of study (7 day) analyses reveal food consumption was comparable in all dose groups.

Necropsy : No necropsy findings related to the test article was observed in this study.

Histopathology : Microscopic analyses reveal no major histopathologic findings due to test article.

NOEL : Not identified, the study was a dose - ranging study.

2. 5 Day Dose Ranging- Finding Inhalation Toxicity Study with R and S Salbutamol in Rats (study # 051-804, Lot #- not given, August 29, 1994).

GLP Statement : No

Laboratory :

Animal Species/ Strain : Wistar Han-ibm (outbred) SPF

Number of Animals : 5/sex

Formulation : Salbutamol dissolved in distilled water and generated by nebulization, 80-90% of the particles was approximately [redacted] µM in size.

Route of Administration : Nose only inhalation

Frequency and Length of the Study : 1 hour daily/5 days

Dose Design :

Groups	**Target Dose Level** (mg/kg)	**Estimated Achieved Dose** (mg/kg)
R-Salbutamol	0.1	0.11
R-Salbutamol	0.5	0.56
R-Salbutamol	5.0	5.73
S-Salbutamol	5.0	5.61

Results :

Mortality : No deaths occurred in this study.

Clinical Observations : No clinical signs were observed in the rats treated with Salbutamol.

Body weight : Body weights were comparable in all dose groups in this study.

Food Consumption : Food consumption was comparable in all dose groups.

Lung Weights :There were no biologically significant effects in the lung weights of any of the rats in any dose group.

Macroscopic Findings : Macroscopic findings were limited to dark red foci in the lungs of a few animals in each dose group.

3. 28 Day Repeat Dose Inhalation Toxicity Study with R-Salbutamol, S-Salbutamol and R-S Salbutamol in Rats (study # 051-805, lot # 022/174, January 31, 1995).

GLP Statement : Yes

Laboratory :

Animal Species/Strain : Wistar, Han-ibm (outbred) SPF

Number of animals : 10/sex

Formulation : Salbutamol was dissolved in distilled water and generated by nebulization. Approximately 80-90 % of the drug particles was μM or less.

Route of Administration : Nose only inhalation

Frequency and Length of Exposure : 1 hour daily/7 days/ week/ 28 days.

Dose Design :

Groups	**Dose Level (mg/kg)**	
	Target	**Achieved**
R-Salbutamol	0.06	0.012
	0.6	0.3
	6.0	3.3
S-Salbutamol	0.06	0.10

	0.6	0.3
	6.0	3.0
R/S-Salbutamol	12	6.4
Vehicle (distilled Water)	-	-

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Results :

Mortality : There were no deaths that could be attributed to Salbutamol.

Clinical Observations : There were no clinical signs observed in rats treated with Salbutamol.

Body Weight : At termination, body weight gain in females and males rats in the R/S Salbutamol dose group increased by approximately 8 and 16 %, respectively. Body weight gain was also increased in the females in the high dose R-Salbutamol group by approximately 6 %.

Food Consumption : At termination, the food consumption was comparable in all dose groups.

Hematology :There were no significant changes in the hematological parameters in the rats treated with Salbutamol.

Clinical Chemistries : There were increases in plasma potassium concentrations in the rats treated with R/S Salbutamol and in the rats treated with R-Salbutamol, 3.3 mg/kg. The increases were approximately 10-17 %.

Urinalysis : There were no findings that could be attributed to R, S or R/S Salbutamol.

Ophthalmoscopic Examination : No treatment-related changes were reported.

Organ Weights : Increases were found in the heart weights of the rats treated with R/S Salbutamol as well as the rats in the high dose R-Salbutamol group. Increases in heart weights were approximately 15 %.

Histopathology : There were no histopathological changes when the tissues of the rats in the high dose group were analyzed using

NOEL: R -Salbutamol : 0.3 mg/kg

4. 28 Day Oral toxicity Study in the Rat (study # 051-801, lot # 022/146, February 25, 1997).

GLP Statement : Yes

Laboratory :

Animal Species/Strain : Rat, Sprague-Dawley

Number/Animals/Dose: 10/sex

Formulation : The drug was dissolved in % methylcellulose.

Route of Administration : Gavage

Frequency and Duration of the Study : The drug was given once daily for 28 days.

Dose Design :

Group	Dose (mg/kg)
Vehicle (methylcellulose)	0
R-Salbutamol	2.5
S-Salbutamol	2.5
R-Salbutamol	12.5

S-Salbutamol	12.5
R-Salbutamol	25
S-Salbutamol	25
R/S Salbutamol	25
R/S Salbutamol	50

Results :
Mortality : None of the animals died during the study.

Clinical Observations : Dose-related alopecia was observed in all dose groups including the vehicle group.

Body weight : Terminal body weights were comparable among all the animals in this study.

Food Consumption : At the end of the study, there were no significant differences in the food consumption among the animals in this study.

Ophthalmology : There were no changes in the eyes of the rats in this study.

Hematology : Terminal analyses reveal that there were no changes in the hematological parameters.

Clinical Chemistries : Terminal analyses revealed that there were no differences in clinical chemistries when the vehicle group was compared with the salbutamol -treated groups.

Urinalyses : Analyses on day 29 reveal no differences in the urinalyses between the control and the treated groups.

Organ Weights : There were no significant increase in the organ weights of the rats in this study.

Necropsy : Changes were found in the spleens of rats in all dose groups. The changes consisted of grey areas on the surface near the tail end, yellow areas on the glandular mucosa and focal area of granularity in the middle portion There were no changes observed in the spleens of the rats in the vehicle group.

Histopathology : Multi-focal treatment-related capsulitis and/or capsular thickening of the spleen was found in all dose groups. There was accumulations of mononuclear cells adjacent to the capsule. These changes were more severe in the males in the R/S Salbutamol and R Salbutamol groups than in the males in the S- Salbutamol group.

NOEL : NoT identified due to unexplained findings in the spleen.

5. Subchronic (92 day) Repeated Dose Inhalation Toxicity Study with R- Salbutamol and R/S Salbutamol in Rats with a 28 Day Recovery Period (study # 051-806, lot # H 765-45 (R-Salbutamol) and (S940701 (R/S Salbutamol), April 3, 1997).

GLP Statement : Yes

Laboratory :

Animal Species/ Strain : Rat, Wistar, Han-ibm (outbred) SPF

Number/Animal/Dose : 10/sex (main study): 5/sex (recovery study).

Formulation : Drug products dissolved in distilled water, placed in nebulizers. 80-90% of the particles were approximately µM in size.

Route of Administration : Nose-only oral inhalation.

Frequency/Length of Study : 1 hour/daily/7 days/weekly / 92 days.

Dose Design :

Groups	Target Dose (mg/kg)	Estimated Dose Level (mg/kg)
R-Salbutamol	0.06	0.057
R-Salbutamol	0.6	0.613
R-Salbutamol	6.0	6.001
R/S Salbutamol	12.0	11.8

Results :
Mortality : 2 rats died, neither of these deaths were drug-related.

Clinical Observations : Salivation was observed in the rats in the high dose R- Salbutamol and the R/S Salbutamol dose group.

Body Weight : On day 90, there were treatment-related increases in the body weight gain in the rats in the high dose R-Salbutamol and R/S Salbutamol dose groups. The increases were greater in females than in males and the increases were approximately 15%. The greatest increases were observed in the rats in the high dose R-Salbutamol.

Food consumption : Food analyses on day 90 show increases in food consumption in the rats in the high dose R-Salbutamol group as well as the R/S Salbutamol group. The increases were approximately 6 %.

Ophthalmoscopy : Terminal examinations reveal no treatment-related effects to the eyes of rats exposed to R-Sabutamol and R/S Salbutamol.

Hematology : At sacrifice, there was increase in the reticulocyte count in the rats in the high dose R-Salbutamol and the R/S Salbutamol group. The increases were approximately 15%.

There were also increases in hemoglobin concentrations in the rats in all R-Salbutamol treated groups as well as the R/S salbutamol group. The increases were approximately 7%.

After a 28 day recovery period, the reticulocyte counts and the hemogloblin concentrations in these rats were within normal limits.

Clinical Chemistries : At sacrifice, dose-related increases in potassium concentrations was found in all R Salbutamol treated rats as well as the rats in the R/S Salbutamol group. The increases were approximately 13-20 %. At the end of the 28 day recovery period, the potassium concentrations were within normal limits.

Urinalysis : At sacrifice, there were no significant changes that were drug-related.

Organ Weights : There were dose-related increases in the mean heart weight of all R-Salbutamol treated rats as well as the rats with R/S Salbutamol. The increases were approximately 2-28%. The increases were greater in the R Salbutamol -treated rats than in the R/S Salbutamol. There were also increases in the mean lung and kidney weights of the rats. The mean lung increases were more pronounced in the female rats and they were dose-related. (approximately 6-15 %). The increases in the mean kidney weights were approximately 2-47 %.

At the end of the 28 day recovery period, mean heart weights were elevated in the rats in the high dose R-salbutamol group as well as the R/S Salbutamol group. The findings suggest that these effects are not reversible. The mean lung and kidneys weights were within normal range at the end of the 28 day recovery period.

Macroscopic Findings : Macroscopic findings were comparable in the control and treated rats.

Histopathology : Focal/multi-focal myocardial necrosis was noted in all R Salbutamol and R/S Salbutamol rats. These findings were characterized by inflammatory cells infiltration, myocardial fibrosis, indicating healing of myocardial tissues, was also noted in these animals. These findings were minimal to slight in the low and mid R- Salbutamol treated rats, moderate to severe in the high dose R-Salbutamol treated group as well as the R/S Salbutamol treated rats. These finding were irreversible as indicated by the data from the 28 day recovery period.

NOEL : There was no NOAEL because there were on the cardiovascular toxicity in all R and racemic albuterol dosed rats..

6. 90 Day Oral Toxicity Study in the Rat with a 28 Day Recovery Period. (Study # 051-802, lot # 007/94 (R-Albuterol), lot # S 921101-C (R/S Albuterol, August 24, 1995).

GLP Statement : yes

Laboratory

Animal species/ strain : Rat, Sprague-Dawley,-Crl-CD (SD).

Number/Animal/Dose : Control and high doses : 30/sex, low and mid dose : 20/sex.

Formulation : Drug dissolved in methylcellulose.

Route of Administration : Oral, gavage

Frequency and Duration : Drug given once daily for 90 days.
Dose Design :

Group	Number Animal /Dose (Male)	(Female)	Dose Level (mg/kg)

Vehicle control	20*	20*	0
R-albuterol	20*	20*	2.5
R-Albuterol	20*	20*	12.5
R-albuterol	30*	30*	25
R/S-Albuterol	30*	30*	50

* Includes 5/sex for the recovery period.

Results :

Mortality : There were no mortalities in this study.

Clinical Observations : There were no clinical signs of systemic toxicity in this study.

Body Weight : At sacrifice, body weight gain was comparable in all dose groups.

Food Consumption : Evaluations at the conclusion of this study, reveal no significant differences in the food consumption in drug or vehicle-treated rats.

Ophthmoscopy : Termination examinations show that that there were no drug-related effects on the eyes of the rats in this study.

Hematology : At sacrifice, there were no drug-related effects observed in the hematology parameters.

Clinical Chemistries : At termination, clinical chemistries were within normal range for all rats in this study.

Urinalysis : Post dosing evaluations, show no significant changes in the urinalysis parameters.

Organ weights : Statistically significant increases in the mean absolute liver weights of females rats in the 12.5 and 25 mg/kg as well as the 50

mg/kg dosed male rats were found. The increases were 19-44 % of the mean absolute liver weight of the rats in the control group.
There were increases in the mean spleen weights of all R-Albuterol - treated rats as well as the rats in the R/S albuterol rats (6-13 %).
The absolute liver weights were within normal range after the 28 day recovery period. The mean spleen weights were still increased after the 28 day recovery period.

Necropsy : There were no significant changes observed when the tissues were examined at necropsy.

Histopathology : Microscopic evaluations reveal changes in the spleens and hearts of all albuterol -treated rats. The changes in the spleen include multi-focal capsulitis, multi-focal capsular thickening and capsular cysts. These findings were greater in males than females. The myocardial changes consisted of multi-focal areas of fiber degeneration and mononuclear cell infiltration (multi-focal non-suppurative myocardial). The changes in the myocardium were greater in males than females. The spleen and myocardial changes were not reversible after the 28 recovery period.

NOEL : None

7. Preliminary Inhalation Study in Dogs with R -Albuterol (study # 051-811, 1997).

The objective of this study was to choose doses for the 7 day inhalation study.

GLP statement: Yes

Laboratory :

Animal strain : Dog, Beagle

Number/ Dose : 2/sex, days 1-25, On day 37, 3 M/4 F, R albuterol and 7 M and 6 F, R/S -albuterol.

Doses : Day 1 : Vehicle
Day 2 : 2 mg/kg
Day 5 : 2 mg/kg
Day 9 : 3 mg/kg
Day 12: 6 mg/kg
Day 17: 6 mg/kg
Day 23: 2 mg/kg
Day 24: saline
Day 25 : 10 mg/kg
Day 25-37 : 13 and 19 mg/kg

The wash out period up to day 12 was 2/3 days, then 5 days between day 12 and day 17.

Administration : R-albuterol was given by inhalation using an [redacted] nebulizer and face mask. The drug was given over a 10-15 minute dosing period.

Results :
Mortalities : None

Clinical signs : Peripheral vasodilatation, increased force of heart rate and marked increases in heart rate and profuse salivation at 6 mg/kg and higher (clinical signs were for R-albuterol and R/S -albuterol).

Necropsy : Forced changes of the endocarial surface of the left ventricular papillary muscles of all dogs.

Toxicokinetics : Blood was taken on day 1 and the day 37, but were not analyzed.

A 7 day Range -Finding Inhalation Toxicity Study in the Beagle Dog with a Nebulizer Aerosol Formulation (Study # 051-815, 1997).

GLP statement : Yes

Laboratory : [redacted]

Animal Strain : Beagle dog

Number/Dose : 2/sex

Doses : Saline vehicle, R-albuterol : 0.001, 0.01, 0.1 and 0.4 mg/kg

Formulation : Lot # 004-0001- drug was prepared in strengths of 0.003-12 mg/mL by dissolving test article in [redacted] sodium chloride.

Administration : Drug was given using an oronasal face mask.

Frequency/ Duration : Once daily for 7 days.

Results :
Mortality : None

Clinical Signs : There were no clinical signs of toxicity in the dogs in this study.

Body Weight : There were no effects on body weights in this study.

Cardiovascular Studies :There was a dose- response tachycardia to R-albuterol up to but not including the 0.4 mg/kg dose. The maximum heart rate occurred between 15 minutes and 2 hours after dosing in the dog.

Macroscopic Findings : In males, smaller testes, epididymides and prostates. In both sex, change of color in the lungs of the dogs.

Histopathology : Slight focus of mineralization in the papillary muscles was found in the dogs treated with R-albuterol, 0.4 mg/kg.

NOAEL : None- the purpose of the study was to obtain data concerning doses for 90 day study.

7 Day Inhalational Dose Finding Study in Dog (study # 051-812, 1997).

GLP Statement : Yes

Laboratory : [redacted]

Animal Species/ Strain : Beagle Dog

Number /Animal/Dose : 1/sex

Doses : Vehicle, 0.1, 0.4 and 2.0 mg/kg (R-albuterol hydrochloride, batch # 004-0001).

Doses :

Drug :	**Estimated Dose (mg/kg)**	**Achieved Dose (mg/kg)**
R-albuterol	0	0
	0.1	0.13
	0.4	0.55
	2.0	2.73

Formulation : Drug was dissolved in saline.

Route of Administration : Inhalation route using [redacted] nebulizer and face mask.

Frequency and Length of Exposure : Drug given once daily for 10-12 minutes for 7 days.

Results :

Mortality : None

Clinical Signs : Increased heart rate and force of heart beat with associated flushing of the skin, particularly the ears, mouth and gums were noted in the R-albuterol treated groups. The most severe clinical signs were noted in the high dose groups. The symptoms lasted for up to 6 hours.

Respiration Rate : No effect on the respiration rate.

Body Weight : Body weight gain was comparable in all dose groups.

Food Consumption : Food consumption was similar in all dose groups.

EKGs : EKGs were taken before the study and on day 7, 2-3 hours after dosing. Results of the EKG tracings reveal peak heart rates between hours 1 and 4. There was a second peak at 5 hours post dosing. The EKG tracings also revealed shorten P-R and Q-T intervals, formation of Ta waves, depression of ST waves, notched QRS complexes and atrial ectopy. Most of these effects were observed in the mid and high dose groups.

Hematology : There was a slight decrease in red blood cells and hemoglobin concentration in all R-albuterol treated dogs.

Clinical Chemistries : There were no major changes in the clinical chemistries in the dog in this study.

Necropsy Findings : Pale foci was observed on the endocardial surface of the ventricle of the dogs in the mid and high dose groups.

Histopathology : Focal myocardial degeneration was found in the myocardium of the dogs in the mid and high dose groups. This effect was characterized by myofiber degeneration (reduction in the size of the and lack of in color in the cytoplasm) associated with fibroblastic and histologic proliferation. Necrotic myocytes were also observed in the myocardial tissues.

Toxicokinetics : Blood was taken before the study and on day 7, however, the blood was not analyzed because an assay for R- albuterol has not been developed.

NOAEL : 0.13 mg/kg

90 Day Oral Inhalation Safety Study in the Beagle Dog With a Nebulized Aerosol Solution (study # 051-816, 1997).

GLP Statement : Yes

Laboratory : [redacted]

Animal species/Strain : Dog, Beagle

Number of Animals : 4/sex

Formulation : Test articles were dissolved in [redacted] sodium chloride. The particle size of the majority of the particles was approximately [redacted] µM.

Route of Administration : Drug was administered using an oronasal face mask fitted with inlet and outlet tubes. The mask was fitted over the dog' s muzzle in such a way that the muzzle is inside the cylinder and the dog is mouth breathing. During the treatment, the dogs were placed in a sling.

Frequency and Duration of the Study : The drug was given once daily, 10 minutes per day and the drug was given for 90 days.

Pharmacokinetics : Dogs were bled pre-dose and on day 90. On day 90, blood was drawn at time periods beginning 5 minutes after dosing until 4 hours after dosing.

Doses : (mg/kg)

	Estimated Dose		Achieved Dose
Drug :	**Male**	**Female**	**Mean**
R-albuterol*	0.002	0.002	0.002
R-albuterol	0.001	0.001	0.001
R-albuterol	0.32	0.45	0.28
R/S albuterol**	0.58	0.45	0.52

*Albuterol hydrochloride
** Albuterol sulfate

Results :

Mortality : There were no deaths in this study.

Clinical Signs : There were no clinical signs observed in this study that were attributed to R-albuterol or R/S albuterol.

Body Weights : At the end of the study, the body weight gain was comparable in all dose groups.

Food Consumption : Food consumption in these dogs were unaffected by treatment with R-albuterol or R/S albuterol.

Ophthalmology : Evaluations at the end of the study divulge that there were no treatment-related ocular changes in the dogs in the study.

EKGs : Tracings were carried out on pre-dose, days 1, 7, 28 and 85. The tracings were carried out 15 minutes to 6 hours after dosing. The results reveal dose-related tachycardia. The tachycardia was evident on all 4 days that the EKG tracings were carried out. The magnitude of the effect decreased during the study suggesting loss of receptor availability and loss of cAMP production.

Hematology : On day 90, there were no changes in the hematology parameters in the albuterol treated dogs.

Clinical Chemistries : On day 90, clinical chemistries were comparable in all the dogs in all dose groups.

Urinalysis : End of the study evaluations show that the urinalysis parameters were unaffected by treatment with R- or R/S albuterol.

Organ Weights : Terminal examinations reveal that organ weights were unaffected by inhaled exposure to R-albuterol or R/S albuterol.

Gross Histopathology : Terminal assessments show no gross changes due to the test articles.

Histopathology : Terminal microscopic analyses revealed that there were no major changes in the tissues of the dogs treated with R- or R/S albuterol. There were several shortcomings in the histopathology evaluations : (1) the low and mid dose tissues were not evaluated microscopically and (2) no special collagen stains were used to help delineate myocardial necrosis (cardiac tissues heal as collagen scars).

Toxicokinetics : Toxicokinetic analyses were not carried out because the sponsor has not been able to develop a reliable assay for R-albuterol.

Summary and Evaluation of the Subchronic Studies :
Subchronic toxicity studies for R-albuterol were carried out in the rat and the dog. The oral and inhalation subchronic studies in the rat were 28 and 90 days. There also was 5 and 7 day dose- ranging studies in the rat. In the 28 day study in the rat, nose only inhalation doses achieved were 0.12-3.3 mg/kg with R-albuterol, 0.10-3.0 mg/kg with S-albuterol and 6.4 mg/kg with R/S albuterol. Results of this study reveal no histopathological changes due to R-albuterol, however, only the tissues of the rats in the high dose groups of R-, S- and R/S albuterol were examined microscopically. In spite of that, the NOAEL for R-albuterol was 0.3 mg/kg because of increases in mean heart weights (initial sign of toxicity) in the rats in the high dose of R-albuterol. Similar findings were found in the rats

treated with the R/S albuterol. In the 28 day oral toxicity study, doses used for R- and S- albuterol were 2.5-25 mg/kg and R/S albuterol dose was 50 mg/kg. Multi-focal treatment- related capsulitis and/or capsular thickening of the spleen was found in all dose groups. Again , only the high dose rat tissues were examined microscopically. There was no NOAEL in this study.

The 90 day nose only inhalation toxicity study in the rat utilized doses (estimated) of R-salbutamol of 0.057, 0.0613 and 6.001 mg/kg and 11.8 mg/kg for R/S salbutamol. All R-salbutamol and R/S salbutamol dosed rats in this study had increases in mean heart weights. The heart tissues of these rats, examined microscopically, were characterized by dose-related focal/multi-focal necrosis with inflammatory cell infiltration. These myocardial tissues contained myocardial fibrosis indicating healing of the tissues. These animals also had increases in mean lung and kidney weights, however, after the 28 day recovery period, mean lung and kidney weights were within the normal range for these rats. There was no NOAEL in this study. In the 90 day oral toxicity study in the rat, gavaged doses of R-albuterol were 2.5-25 mg/kg while the dose of R/S albuterol in this study was 50 mg/kg. These drugs induced dose-related changes in the spleen and heart tissues. The spleen tissues included multi-focal capsulitis, multi-focal capsular thickening and capsular cysts. The heart changes consisted of multi-focal areas of fiber degeneration and mononuclear infiltration. These changes were greater (number) and more severe in males than females. The male rat is more sensitive to the effects of beta adrenergic agonists than females because of the difference in weight (males are heavier than females). These changes were irreversible. There was no NOAEL in this study. The low dose, 0.057 mg/kg in the study induced myocardial toxicity in the rat. The maximum recommended daily inhalation dose is 0.075 mg/kg.

There were 4 subchronic studies in the dog, 3 were preliminary dose ranging studies and the studies will not be discussed here. The main study in the dog was a 90 day subchronic inhalation toxicity study. The achieved doses in the beagle dog was 0.002, 0.001 and 0.28 mg/kg, R-

albuterol and 0.52 mg/kg , R/S albuterol. The drug was delivered via oranasal face mask.
Results of the study revealed that R-albuterol and R/S albuterol induced dose-related tachycardia at all dose levels. The myocardial tissues of the R-albuterol high dose dogs and the R/S albuterol treated dogs, examined microscopically revealed no major changes in these tissues. However, the myocardial tissues of the dogs in the low and mid dose groups were not evaluated microscopically. The NOAEL for R-albuterol was thought to be 0.28 mg/kg.

There were no pharmacokinetic/toxicokinetic data in the subchronic studies. The sponsor has not develop a reliable assay for R-albuterol.

Reproduction Study :
Study for Effects on Embryo-Fetal Development in Rabbits (study # 051-807, 1996, amended 1997).

Laboratory :

Animal Species/ Strain : Rabbits, white, New Zealand

Number of animals/Dose: 19 except the control group which consists of 20 animals.

Formulation : Lot # 0030002 (R-albuterol), S-940701 (R/S-albuterol) and 022/176 (S-albuterol). Drug dissolved in CMC.

Administration : Oral gavage, drug given once daily, day 6-20 of gestation.

Dose Design :

Drug	**Dose Level (mg/kg)**	**Number/Females**
Control	CMC*	20
R-albuterol	0.5	19
R-albuterol	5.0	19

R-albuterol	25	19
R/S	50	19
S-albuterol	25	19

* = carboxymethylcellulose

Results :

Mortality : There were 3 deaths in the control group, as well as one in the 5.0 mg/kg and I in the 25 mg/kg dose group. The deaths were attributed to mechanized injuries. There were no drug-related deaths.

Clinical Signs : Several does had excess salivation and labored respiration.

Body Weights : Body weight gain was comparable in all dose groups.

Food Consumption : There were no differences in the food consumption of the rabbits in this study.

Cesaean Section Data : On day 29, there were no meaningful biological differences between the vehicle control and the albuterol groups (R-, S- and R/S -albuterol) in cesarean section parameters. Parameters measured included the numbers of corpora lutea, implantations sites, viable and nonviable fetuses, early and late resorptions, pre-implantation losses, post-implantation's, total loss/liter, fetal sex ratio and gravid uterus and fetal weights.

Fetal Morphological Observation : On day 29, there were no treatment-related malformations or developmental variations observed in the fetuses of the rabbits in any dose group.

Summary of the Reproduction Study :
A teratology study was carried out to determine the effects of R-, S - and R/S albuterol on embryo-fetal development in New Zealand white rabbits. R-albuterol, oral doses of 0.5, 5.0 and 25 mg/kg, S-albuterol, oral dose of

25 mg/kg and R/S-albuterol, oral dose of 50 mg/kg were administered on rabbits day 6-20 of pregnancy. R-, S and R/S-albuterol, given orally did not induce embryo or fetal toxicity at the doses tested in this study.

Genotoxicity Assays :

1. Ames /Salmonella -E-coli Reverse Mutation Assay on R-, S- and R/S Albuterol (study # 051-808, 1996).

Laboratory : [redacted]

R-, S- and racemic albuterol was evaluated in the Ames Salmonella-E-coli reverse rmutation assay to determine the ability to induce reverse mutation at selected histidine loci in five test strains of Salmonella typhimurium (TA 1535, TA 1537, TA98, TA100, and TA 102) and at tryptophan locus in Escherichia coli test strain (WP2uvrA) Both assays were conducted in the presence and absence of S_9.

The strains were exposed to 50-10,000 μg/plate. The results indicate that R-albuterol demonstrated a non-reproducible 2.2 fold increase in reversion frequency in the liquid preincubation assay in strain TA 100 with S_9 only. R/S -albuterol demonstrated a non-reproducible 2.1 fold increase in reversion frequency in plate incorporation assay in strain TA 1537 with S_9 only. However, these increases were not statistically significant nor dose dependent and were not reproducible upon retesting. S-albuterol was reproducibly negative in each assay.

2. CHO/HPRT Mammalian Forward Gene Mutation Assay On R-, S- and R/S Albuterol (study # 051-810, 1997).

Laboratory : [redacted]

R-, S- and R/S albuterol were evaluated in the CHO/HPRT mammal cell forward gene mutation assay in order to determine their ability to induce mutations at the hypoxanthine-guanine phosphoribosyl transferase (HPRT) in cultured Chinese hamster ovary (CHO) cells. The cells were incubated at 10 concentrations (0.167-5000 μg/mL) in the presence and

absence of S_9. Results of this study show these albuterol compounds to be cytoxic at the 5000 μg/mL concentrations. Statistically significant increases in average mutant frequencies, to approximately 4.1-14 fold of the negative control values, were observed in cells treated with R-, S-and R/S albuterol in one or more concentrations in the presence and absence of S_9. However, none of these increases represented a net increase of 20 mutants/106 clonable cells and all average mutants frequencies were considered to be within acceptable negative control ranges.

Summary of the Genotoxicity Studies :

Two in vitro genotoxicity assays were carried out in an attempt to further determine the mutagenic potential for R-albuterol. Ames /Salmonella-E-coli Reverse Mutation assay and CHO/HPRT Mammalian forward gene mutation assay were investigated in the in the presence and absence of S_9.

The concentrations in these assay was 5000-1000 μg/plate. R-, S - and racemic albuterol were negative in these assays at the concentrations tested.

Metabolism :

Metabolism studies were carried out in dog, rat, guinea pig and rabbit after oral administration of racemic albuterol. The results of these studies are listed below :

Study	Species	Results
Comparison metabolic pathways for various species. (study # 051-450B)	dog rat guinea pig rabbit	Dog did not metabolize racemic albuterol. The rat, guinea pig and rabbit completely metabolized racemic albuterol to a glucuronic acid conjugate. The drug was given orally.

Assessing the metabolism of ^{3}H-albuterol in animal species. (study # 051 450).	rat guinea pig rabbit dog	There was no stero--selective in animals. A single dose of labeled (1μCi/kg) ^{3}H-albuterol was administered (3 mg/kg) orally to rats, guinea pigs, rabbits and dog. Urine and feces were collected for 24 hours. No data were submitted.

Summary of the Metabolism Studies :

Two preliminary studies were carried out to investigate the metabolism of albuterol in several animal species, rats, dogs, guinea pigs and rabbits. The dog did not metabolize racemic albuterol after oral administration, however, rats, guinea pigs and rabbits metabolize oral racemic albuterol to a glucuronic acid conjugate. The was no evidence of steroselectivity in any of these animal species. Data has been published that suggest that racemic albuterol is stereselective in favor of R-albuterol in humans. These data suggest that man metabolizes albuterol differently than the dog and perhaps, the rat, guinea pig and rabbit.

The animal studies did not include data on R-, or S- albuterol because of unreliable assays for these isomers. However, the sponsor feels that they have developed a reliable assay for R-albuterol-albuterol in the dog.

Labeling Recommendations :

The information in the preclinical section in the labeling should be expunged from the label for Sepracor. Preclinical data in this submission do not uphold the information contained in the preclinical section. Additionally, even if the information was supported by in vitro and in vivo data in animals, clinical significance is unknown. The label is for patients, 12 years and older.

The preclinical labeling for Sepracor should be amended as follows :
Preclinical :
Clinical Pharmacology Section:
Intravenous studies in rats with albuterol sulfate have demonstrated that albuterol crosses the blood brain barrier and reaches brain concentrations amounting to approximately 5.0 % of the plasma concentrations. In structures outside the brain barrier (pineal and pituitary glands), albuterol concentrations were found to be 100 times those in the whole brain.

Studies in laboratory animals (minipigs, rodents and dogs) have demonstrated the occurrence of cardiac arrhythmias and sudden death (with histologic evidence of myocardial necrosis) when beta agonists and methylxanthines are administered concurrently. The clinical significance of these findings is unknown.

Carcinogenesis, Mutagenesis, and Impairment of Fertility: No carcinogenesis or impairment of fertility studies have been carried out with However, albuterol sulfate has been evaluated for its carcinogenic and impairment of fertility potential.
In a 2-year study in Sprague-Dawley rats, albuterol sulfate caused a significant dose-related increase in the incidence of benign leiomyomas of the mesovarium at and above dietary doses of 2 mg/kg/day (approximately 2 times the maximum recommended daily inhalation dose of for adults on a mg/m^2 basis). In another study this effect was blocked by the coadministration of propranolol, a non-selective beta-adrenergic antagonist. In an 18-month study in CD-1 mice, albuterol showed no evidence of tumorigenicity at dietary doses up to 500 mg/kg/day (approximately 270 times the maximum recommended daily inhalation dose of for adults on a mg/m^2 basis). In a 22- month study in the Golden hamster, albuterol sulfate showed no evidence of

tumorigenicity at dietary doses up to 50 mg/kg (approximately 35 times the maximum recommended daily inhalation dose of [redacted] for adults on a mg/m^2 basis).

[redacted] demonstrated no evidence of impaired fertility at oral doses up to 50 mg/kg (approximately 55 times the maximum recommended daily inhalation dose of [redacted] for adults on a mg/m^2 basis).

Teratogenic Effects--Pregnancy Category C: A reproduction study in New Zealand white rabbits revealed [redacted] was not teratogenic when administered orally at doses up to 25 mg/kg (approximately 110 times the maximum recommended daily inhalation dose of [redacted] for adults on a mg/m^2 basis). R-S albuterol sulfate [redacted] has been shown to be teratogenic in mice. A study in CD-1 mice at subcutaneous (sc) doses at and above 0.25 mg/kg (less than the maximum recommended daily inhalation dose of [redacted] for adults on mg/m^2 basis) showed cleft palate formation in 5 of 111 (4.5%) fetuses. At a subcutaneous dose of 2.5 mg/kg (approximately [redacted] maximum recommended daily inhalation dose of [redacted] for adults on a mg/m^2 basis) showed cleft formation in 10 of 108 (9.3%) fetuses. The drug did not induce cleft palate formation when administered at a subcutaneous dose of 0.025 mg/kg (less than the maximum recommended daily inhalation dose of R-albuterol on a mg/m^2 basis). Cleft palate also occurred in 22 of 72 (30.5%) fetuses from females treated with 2.5 mg/kg isoproterenol (positive control) administered subcutaneously [redacted] A reproduction study in Stride

Dutch rabbits revealed cranioschesis in 7 of 19 (37%) fetuses when albuterol sulfate was administered orally at 50 mg/kg (approximately 110 times the maximum recommended daily inhalation dose of for adults on a mg/m^2 basis).

There are no adequate and well-controlled studies in pregnant women. should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus.

During marketing of various congenital anomalies, including cleft palate and limb defects, have been reported in the offspring of patients being treated with Some of the mothers were taking multiple medications during their pregnancies. Because no consistent pattern of defects can be discerned, a relationship between albuterol use and congenital anomalies has not been established.

Overdosage :
The IV median lethal dose of in mice is approximately 66 mg/kg (approximately 70 times the maximum recommended daily inhalation dose of for adults on a mg/m^2 basis).
The inhalation median lethal dose has not been determined.

Drug:

	age	mg/dose	# daily doses	mg/day	kg	mg/kg	factor	mg/m²
Adult	>12	1.25	3	3.75	50	0.08	37	2.78

	route	mg/kg/d	conv. factor	mg/m²	Dose Ratio Adults	Rounded Dose Ratio Adults
Carcinogenicity:						
mouse			3	0	—	—
mouse			3	0	—	—
mouse	dietary	500	3	1500	540.541	540*
rat	dietary	2	6	12	4.31	4.0*
hamster	dietary	50	4	200	71.9	70
Reproduction and Fertility:						
rat	oral	50	6	300	108.108	110*
rat			6	0	—	—
rat			6	0	—	—
extra			—	—	—	—
Teratogenicity:						
mouse	sc	0.25	3	0.75	0.27027	¼*
mouse	sc	0.025	3	0.075	0.0270	¼*
rabbit	oral	25	12	300	108.108	110
rabbit		50	12	0	215.8—	220*
mouse	sc	2.5	3	7.5	2.7027	3*
Overdosage:						
mouse	iv	66	3	198	71.3514	70
mouse			3	0	—	—
rat			6	0	—	—
rat			6	0	—	—
Other: (Describe studies here)						
rat			6	0	—	—
rat			6	0	—	—
mouse			3	0	—	—
mouse			3	0	—	—
extra			—	—	—	—

* Calculations in labeling are ½ of the dose ratios based on the fact that R-albuterol is 50% of the racemic mixture of albuterol sulfate.

Overall Summary and Evaluation :

[redacted](levalbuterol HCL) composed of R-albuterol isomer only, (racemic albuterol consists of equal isomers of R and S) is a beta adrenergic agonist which is to be administered by inhalation and used in the treatment of and/or the prevention of [redacted]bronchospasm in patients. The NDA was submitted as a 505 (b)(2) meaning that the sponsor can rely on data from other marketed NDAs for racemic albuterol. During a meeting between the sponsor and the FDA, it was agreed that bridging studies would be necessary to support the safety of R-albuterol. These bridging studies would consist of two 28 day studies in a rodent and non-rodent species and then a 90 day studies in the animal species that was found to be the most sensitive to R-albuterol in the 28 day studies. The FDA also recommended a teratology study to determine the effects of R-albuterol on embryo-fetal development .

Twenty eight day and 90 day subchronic studies were carried out in rat as well as a 90 day study in the dog. In the 28 and 90 day studies in the rat, R-albuterol was administered orally (doses 2.5-25 mg/kg) and by inhalation (0.3-6 mg/kg). Racemic albuterol was included in these studies as the positive control.

The target organs in these studies was the cardiovascular system and the spleen. Toxicities in the cardiovascular system included tachycardia, increases in mean heart weight and myocardial necrosis. Spleen toxicity consists of multi-focal capsulitis and multi-focal capsular thickening. The NOAEL in the 28 day inhalation studies was approximately 0.3 mg/kg. There were no NOAELs in the 90 day inhalation or oral subchronic studies in the rat.

In the 90 day inhalation study in the dog, R-albuterol at doses of 0.02-0.28 mg/kg induced tachycardia. The NOAEL in this study was determined to be 0.28 mg/kg, however, it seems unlikely that significant increases in heart rates for periods of 4-6 hours would not result in injuries to the myocardial tissues. The use of a special collagen stain and the

microscopically evaluation of the tissues of all the dogs would have provided more reliable, accurate data which could be used to more closely delineate the NOAEL in this study. We really don't know whether there were any myocardial changes in the mid and low dose groups. Further, we do not have data concerning the drug plasma levels, or the C_{max} or AUCs because of the lack of a assay for R-albuterol. However, the sponsor could have obtain pharmacokinetic parameters for racemic albuterol and then estimated the parameters for R-albuterol.
There were several shortcomings in these studies. (1) only the tissues of the animals in the high dose and the control groups were examined microscopically in most studies. (2) no special collagen stains were used to more clearly delineate myocardial necrosis (3) the sponsor has not been able to develop a reliable, accurate assay for R-albuterol, therefore, it was not possible to correlate drug plasma levels and toxicity (toxicokinetics). (4) there are no data on whether R-albuterol is converted to S-albuterol and visa versa. As a result of these shortcomings, the NOAEL for R-albuterol was not clearly delineated in the rat and the dog in the 90 day studies. It is difficult to delineate a NOAEL for albuterol in animals, therefore, it is very important and necessary to utilize special stains and evaluate the cardiac tissues (papillary muscles) of all the animals in each dose group.

The preclinical data submitted for Sepracor in this NDA submission confirms that the toxicological profile for R-albuterol and racemic albuterol are similar. The toxicity studies for R-albuterol and R/S albuterol reveal that the major target organ for these drugs is the heart. Additionally, pharmacology studies show that R- and racemic albuterol are similar therapeutically. Genotoxicity assays also show that these drugs are not mutagenic. Actually, the sponsor was not required to do the mutagenic assays and if they had discussed their plans with us for their mutagenesis studies, we would have recommended that they include an in vivo mutagenic assay as part of their studies. In a teratology study in the rabbit in which oral doses up to 25 mg/kg were administered, R-albuterol was not teratogenic. Previous reproduction study data in Stride Dutch rabbits revealed cranioschisis in 7 of 19 (37%) fetuses when albuterol was

administered at oral doses of 50 mg/kg . Albuterol was also found to be teratogenic in mice at subcutaneous dose of 0.25 and 2.5 mg/kg. It is unclear in the study submitted by the sponsor whether the drug was administered by the route that provides the greatest systemic esposure.

The pharmacology, toxicology, teratology and genotoxicity studies show that R-albuterol and R/S albuterol are similar and therefore the NDA is approvable. The NDA approval is based mainly on the preclinical and clinical data previously submitted for racemic albuterol.

Conclusion : NDA may be approved from the standpoint of pharmacology.

Recommendation : Please send labeling revisions to the sponsor .

/S/

Virgil Whitehurst 5-12-98
Pharmacologist

/S/ 5/12/98

CC : Div File
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